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Epigallocatechin-3-gallate and Bcl-2 family inhibitor synergize to inhibit glioblastoma cell proliferation and migration

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Abstract

Objective: Shifts in the balance of Bcl-2 family proteins are known to contribute to tumor cell resistance to ABT-737. The present research therefore investigated the role of epigallocatechin-3-gallate(EGCG) in modulating Mcl-1 expression and its subsequent impact on the sensitivity of glioblastoma cells to ABT-737.

Materials and Methods: To assess treatment effects, we conducted a series of assays. Cell proliferation was measured using trypan blue exclusion and colony formation assays, while cytotoxicity was determined via the MTT assay. We evaluated cell migration with a wound healing assay and analyzed apoptosis through Hoechst 33342 staining, an ELISA cell death assay, and a caspase-3 activity assay. Additionally, *Mcl-1* mRNA expression levels were quantified using qRT-PCR.

Results: The results showed that combination therapy with EGCG and ABT-737 significantly enhanced the potency of ABT-737, as evidenced by a marked reduction in its IC_{50} value. This treatment also synergistically suppressed colony formation, migration, survival, and growth of glioblastoma cells more effectively than either EGCG or ABT-737 alone. Specifically, EGCG treatment was found to significantly inhibit *Mcl-1* mRNA expression. This downregulation of *Mcl-1* was further associated with increased apoptosis induction by ABT-737.

Conclusion: EGCG exerts multiple anti-tumor effects, such as inhibiting colony formation, proliferation, and migration, while also activating the intrinsic apoptosis pathway. Furthermore, it can sensitize glioblastoma cells to ABT-737, an effect associated with the suppression of *Mcl-1* mRNA expression.

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Introduction

Glioblastoma multiform (GBM) is the most prevalent and aggressive primary brain cancer in humans (Haar et al. 2012; Taylor et al. 2019). Current treatment strategies for glioblastoma multiform are chemotherapy, radiotherapy and immunotherapy (Afshari et al. 2019; Haar et al. 2012). The efficacy of treatment is severely limited by various chemoresistance mechanisms, resulting in a five-year survival rate of less than 10% (Mousavi et al. 2024; Noch et al. 2018; Shaabani et al. 2020). It is therefore imperative to develop novel therapeutic approaches to counter this resistance.

The Bcl-2 family of proteins is key regulators of the intrinsic apoptosis pathway. Its anti-apoptotic members, which include Bcl-2, Bcl-xL, and Mcl-1, are characterized by four conserved BH domains (BH1-BH4) These specific proteins are frequently overexpressed in various cancers including glioblastoma multiform (Ngoi et al. 2020; Warren et al. 2019). Moreover, elevated levels of these proteins are associated with cell survival and chemoresistance (Ngoi et al. 2020; Warren et al. 2019). Therefore, targeting the anti-apoptotic proteins are attractive therapeutic targets for the development of new therapies for glioblastoma multiform (Chipuk et al. 2006; Ngoi et al. 2020; Warren et al. 2019).

The small-molecule inhibitor ABT-737 targets and inhibits Bcl-2, Bcl-xL, and Bcl-w, and has shown activity against various cancers on its own (Gunda et al. 2017; Li et al. 2009). A significant limitation is its weak binding to anti-apoptotic proteins such as Mcl-1 and Bfl-1/A1. Research has established that high *Mcl-1* expression in cancer cells confers resistance to this drug (Gunda et al. 2017; Kang et al. 2008). Therefore, the combination of ABT-737 with Mcl-1 inhibitors has been proposed as a potential strategy to sensitize tumor cells to ABT-737 (Gunda et al. 2017; Kim et al. 2017; Woo et al. 2017).

Green tea, derived from the leaves of *Camellia sinensis*, accounts for roughly 20% of global tea consumption. Its primary active components are flavonoids and catechins, a class of polyphenols(Jacob et al. 2017). These include epicathechin, epicathechin-3-gallate (ECG), epigallocatechin, and epigallocatechin-3-gallate (EGCG), with green tea being particularly rich in the latter. EGCG is the most abundant catechin consist of green tea that shows antioxidant, anti-inflammatory, anti-bacterial, anti-aging, and anticancer properties (Miyata et al. 2018; Oz 2017; Pervin et al. 2018). Studies have shown that EGCG can suppress the growth and induce apoptosis in various cancerous cells (Miyata et al. 2018; Oz 2017; Pervin et al. 2018). Furthermore, EGCG has been reported to enhance the anti-tumor effects of some chemotherapeutic drugs and molecular-targeted agents (Abe and Inoue 2021; Almatroodi et al. 2020; Shirakami and Shimizu 2018). However, the effect of EGCG on sensitivity of the glioblastoma cells to ABT-737 remains to be elucidated. This study tested the hypothesis that EGCG sensitizes glioblastoma cells to ABT-737 by suppressing *Mcl-1* expression. We therefore investigated the effect of EGCG on both *Mcl-1* levels and cellular sensitivity to the drug.

Materials and Methods

Cell culture

The human glioblastoma cell lines T98G and U373-MG were acquired from the Pasteur Institute in Tehran, Iran. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) from Gibco, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin-streptomycin, 1% sodium pyruvate, and 2 mM glutamine. They were maintained at 37°C in a humidified atmosphere of 5% CO_2. Treatments were administered once the cells reached approximately 80% confluence.

Cytotoxicity Assay

Briefly, cells were seeded into 96-well culture plates at 2×10^3 cells/well. After 24 hr, the cells were treated with various

concentrations of ABT-737 (0-128 µM), and EGCG (0-128 µM), alone and in combination. Dimethyl sulfoxide (DMSO) was used as the solvent for the ABT-737 and EGCG and was used for normalization in all experiments. After 48 hr of incubation, the cytotoxicity of the treatments was assessed by a cell proliferation MTT kit as described by the manufacturer's protocol (Roche Diagnostics GmbH, Mannheim, Germany). Using a plate reader (Awareness Technology, Palm City, FL, USA), absorbance was quantified at 570 nm, using 650 nm as a reference wavelength. The half-maximal inhibitory concentration (IC_{50}), defined as the drug concentration that induced 50% cell death, was computed with GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA). Subsequent experimental treatments were based on these determined IC_{50} values.

Combination effect analysis

The potential synergistic interaction between ABT-737 and EGCG was assessed by calculating the combination index (CI) using the Chou-Talalay method (Ashofteh et al. 2021; Chou and Talalay 1984). The results obtained from the MTT assay were converted into Fraction affected values. A Fa value of 0 represents 100% cell viability while a Fa value of 1 indicates 0% viability. The CompuSyn program from Combosyn (Paramus, NJ, USA) was used to analyze Fa values. A CI value less than 1 indicates a synergic effect, a CI value greater than 1 suggests an antagonistic effect, and a CI value equal to 1 signifies an additive effect.

Cell proliferation assay

Briefly, cells were seeded in 6-well plates at a density of 4×10^4 cells per well and treated with ABT-737 and EGCG, both individually and in combination. The plates were incubated for periods ranging from 24 to 120 hr. At designated time points, the cells were collected and mixed with an equal volume of 0.4% trypan blue solution (Merck KGaA, Darmstadt, Germany). After 3-min incubation, viable (unstained) cells were counted manually using a hematocytometer and an inverted microscope (Nikon Instrument Inc., Melville, NY, USA).

Quantitative real-time PCR (qRT-PCR)

The total cellular RNA extraction from glioblastoma cells was performed after a 48 hr treatment using the RNA extraction kit (Parstous, Tehran, Iran). Reserve transcription of 1µg of total RNA was performed using Easy cDNA synthesis kit (Parstous) and oligo-dT primer according to the manufacturer's protocol. The qPCR protocol was executed in a final volume of 20 µl per reaction. The master mix was prepared with 10 µl of SYBR Green qPCR Master Mix (Parstous), 1 µl of cDNA template, 1 µl of each specific primer, and 7 µl of nuclease-free distilled water.

The oligonucleotides primers used for each target gene are listed below: *Mcl-1*: forward, 5'-GACATCCGCAAAGACCTGTA-3', reverse, 5'-GGAGCAATGATCTTGATCTTCA-3', *β-actin,* forward, 5'-TAGTTAAACAAAGAGGCTGGGA-3', reverse, 5'-CCTTCTAGGTCCTCTACATGG -3', for, and *MMP-2* forward, 5'-ATACCATCGAGACCATGCG-3', and reverse, 5'-CCAATGATCCTGTATGTGATCTG-3. The initial incubation process was carried out at a temperature of 95°C for a period of 10 min. Next, 40 cycles were performed, each with different temperature and duration parameters. The cycles were composed of 10 sec at 95°C, 20 sec at 59°C and 20 sec of 79°C. To access the relative transcript abundance, the $2^{-(\Delta\Delta Ct)}$method (Amri et al. 2022), was utilized, with β-actin serving as the endogenous control.

Wound migration assay

The migratory capacity of glioblastoma cells was investigated using a scratch assay. Briefly, cells were grown

to 90% confluence in 6-well plates. A linear wound was generated in the monolayer using a micropipette tip, followed by two phosphate buffer saline (PBS) washes. The cells were then incubated with the designated treatments, ABT-737 as a single agent or in combination with EGCG, for 48 hr. The extent of cell migration into the scratch was quantified via inverted microscopy.

Clonogenic assay

U373-MG and T98G cells were seeded in 6-well plates at a density of 5×10^3 cells per well and allowed to adhere overnight. The cells were then treated with IC_{50} concentrations of ABT-737 and EGCG, both individually and in combination. Following two-week incubation, the resulting colonies were washed with PBS, fixed with 3.7% paraformaldehyde, and stained with 0.4% crystal violet for 15 min. After a final PBS wash, the colonies were quantified using microscopy.

Cell apoptosis analysis by Hoechst 33342

U373-MG and T98G cells were seeded into a 12-well plate (3×10^3 cells/well) for 24 hr, and treated with ABT-737 and EGCG, alone and in combination. After 48 hr, the cells were washed with PBS, fixed with formaldehyde and stained with Hoechst 33258 (5 µg/ml) (Beyotime, Jiangsu, China) for 15 min. Apoptotic features were assessed by analyzing chromatin condensation or nuclear fragmentation by fluorescent microscopy.

ELISA cell death assay

The extent of apoptosis was measured via a photometric enzyme immunoassay. Cells were plated at a density of 1×10^5 cells per well and exposed to the IC_{50} of the respective treatments for 48 hr. After the incubation period, cells were lysed and the homogenate was centrifuged at 200 ×g for 10 min. A 20 µl aliquot of the supernatant, representing the cytoplasmic fraction of histone-associated DNA fragments, was added to streptavidin-coated wells along with 80 µl of anti-histone-biotin and anti-DNA-POD antibodies. The plate was incubated for 2 hrat room temperature, washed, and developed with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) substrate. The absorbance was quantified at 405 nm, and cell death is expressed as the fold-increase in apoptosiscompared to untreated controls.

Caspase-3 activity assay

In brief, the tumor cells were resuspended in 50 µl lysis buffer and then centrifuged in 10,000 rpm. Subsequently, a volume of 50 µl of 2X reaction buffer, which consists of 10 mM DTT and 5 µl of the 4 mM caspase-3 (DEVD-pNA) colorimetric substrate, were added to each sample. Following a 2 hr incubation period at 37ºC, the absorbance was quantified at 405 nm using an ELISA plate reader. The results are expressed as fold change relative to the control group.

Statistical analysis

All of the quantitative data are presented as mean ±SD of triplicate experiments. The differences between groups were examinedby using one way analysis of variance (ANOVA) followed by Student's *t*-test. p value of <0.05 was considered significant. All statistical analyses were performed using the Prism statistical package (GraphPad, San Diego, CA, USA).

Results

EGCG increased the sensitivity of glioblastoma cells to ABT-737

The MTT assay revealed that both EGCG and ABT-737 significantly suppressed glioblastoma cell survival in a dose-dependent manner ($p<0.05$). As summarized in Table 1, the 48-hrIC_{50} for EGCG was determined to be 87.4 µM in U373-MG cells and 121.8 µM in T98G cells, while the IC_{50} for ABT-737 was 18.6

μM and 35.9 μM, respectively. Critically, the combination of EGCG and ABT-737 produced a synergistic effect, leading to a significantly greater reduction in cell survival and a lower IC_{50} value compared to either agent alone ($p<0.05$). These results collectively demonstrate that EGCG enhances the sensitivity of glioblastoma cells to ABT-737.

	IC_{50} (μM)	
	U373-MG	T98G
EGCG	87.4	121.8
ABT-737	18.6	35.9
Combination	13.8*	23.2*

Table 1. IC_{50} values of the EGCG and ABT-737, alone and in combination, in U737-MG and T98G cell lines.Data are expressed as the mean±SD (n=3). *p<0.05 versus single treatment

The cytotoxic synergic interaction between EGCG and ABT-737 was determined to be synergistic

The interaction between EGCG and ABT-737 in regard of cytotoxicity was evaluated using the Chou-Talalay method for combination index (CI) analysis, which revealed a synergistic effect (CI<1) across a range of concentrations (0–128 μM for each drug), as illustrated by the CI–Fa curves in Figure 1. Our results revealed that the best mean CI value of 48 hr was observed at 64 μM ABT-737 in combination with 64 μM EGCG (CI=0.71) with Fa level of 0.93 in U373-MG cells (Figure 1). Moreover, in T98G cells, the best mean CI value was observed at 128 μM ABT-737 in combination with 128 μM EGCG (CI=0.70) with Fa level of 0.98 (Figure 1).

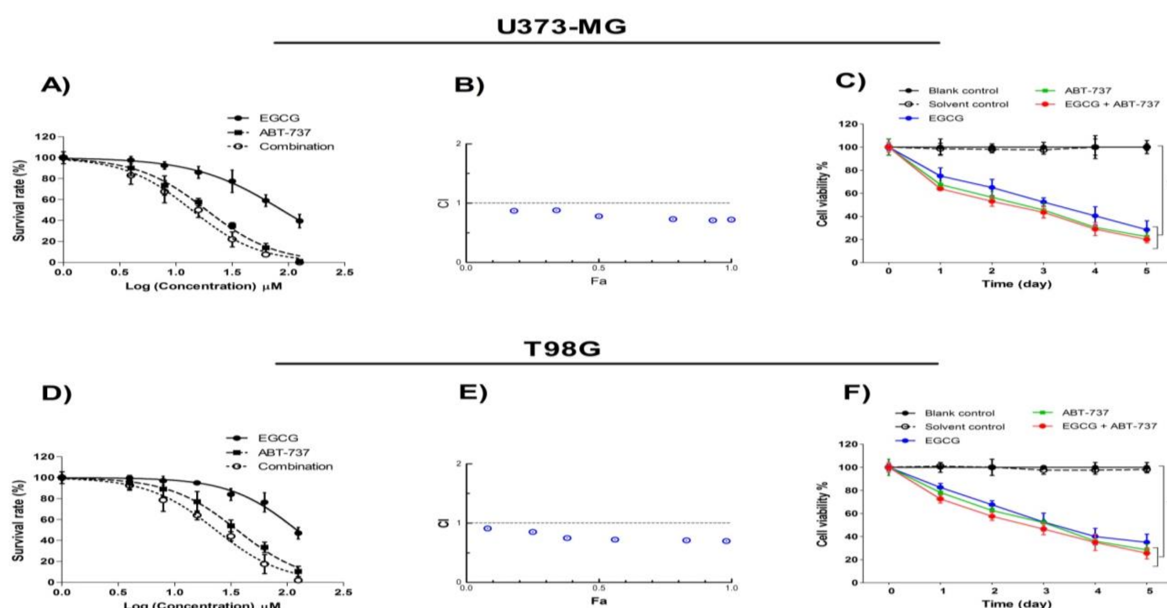

Figure 1. Effect of EGCG and ABT-737 on proliferation and survival of glioblastoma cells. The U373-MG and T98G cells were exposed to the EGCG (A) and ABT-737 (D) at concentrations described in MTT assay. After a 24 hr incubation period, the MTT assay was employed to evaluate the survival rate of the cells. By utilizing the data obtained, GraphPad software was employed to generate cell survival curves (A and D). The mean±SD of three independent experiments is depicted in the data provided within this study. Utilizing CalcuSyn software, the combination index (CI) values were determined by analyzing the Fa values obtained from the MTT assay (B and E). C and F show the proliferation curves of the breast cancer cells. *p<0.05 relative to control groups.

EGCG enhanced the growth-inhibitory action of ABT-737

Time-dependent assays revealed that the combinatorial treatment yielded the most potent anti-proliferative effect. Specifically, in U373-MG cells, the combination reduced viability to 64% at 24 hr, compared to 75% and 67% for EGCG and ABT-737 monotherapy. By the conclusion of the experiment, the

combination had driven viability down to 20%, outperforming the individual treatments (28% and 22% forEGCG and ABT-737, respectively; $p<0.05$; Figure 1). An analogous synergistic effect was confirmed in the T98G cell line. In the combination group, the IC_{50} value was lower than the IC_{50} value in single treatment groups. Therefore, these data suggest that the effect of the combination of two compounds on inhibiting cell proliferation is greater than their individual effect.

EGCG suppressed the expression of apoptotic (*Mcl-1*) and metastatic (*MMP-2*) genes

qRT-PCR analysis revealed that EGCG treatment significantly downregulated the mRNA expression of *Mcl-1* and *MMP-2* compared to the control. In contrast, ABT-737 monotherapy did not notably alter *MMP-2* levels but increased *Mcl-1* expression. The combination therapy resulted in a significant reduction of MMP-2, surpassing the effect of ABT-737 alone, though it was less pronounced than with EGCG alone. Notably, the combination treatment effectively counteracted the upregulation of *Mcl*-1 induced by ABT-737, resulting in a marked difference from both monotherapy and the control. No significant variations were observed between the blank and solvent control groups. These consistent gene expression patterns were confirmed in both U373-MG and T98G cell lines (Figure 2, $p<0.05$).

The anti-migratory effect of ABT-737 on glioblastoma cells was significantly enhanced by EGCG

The wound-healing assay demonstrated that both EGCG and ABT-737 individually inhibited glioblastoma cell migration. Notably, the combination of these agents produced a comparable inhibitory effect at a lower concentration, indicating that EGCG enhances the anti-migratory potency of ABT-737. Quantitative data for wound closure is presented in Figure 3.

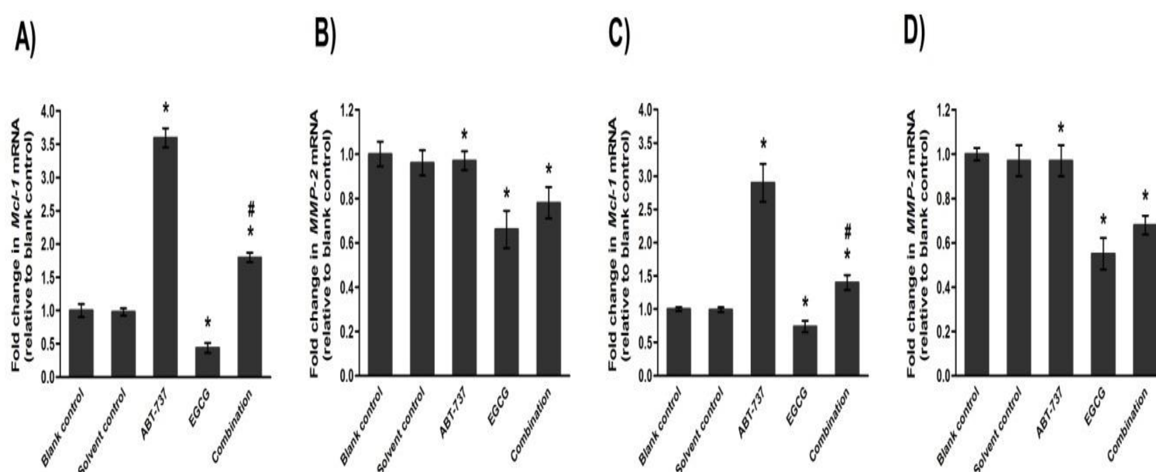

Figure 2. RT-qPCR analysis of glioblastoma cells. After 24 hr of treatment, the RNA was extracted and reversetranscripted to cDNA. The RT-qPCR and $2^{-(\Delta\Delta Ct)}$ method were utilized to evaluate the relative mRNA expression levels of Mcl-1 and MMP-2 in U373-MG (A and B) and T98G (C and D). The results are reported in the form of mean±SD (n=3). #$p<0.05$ relative to monotherapy; *$p<0.05$ relative to control groups.

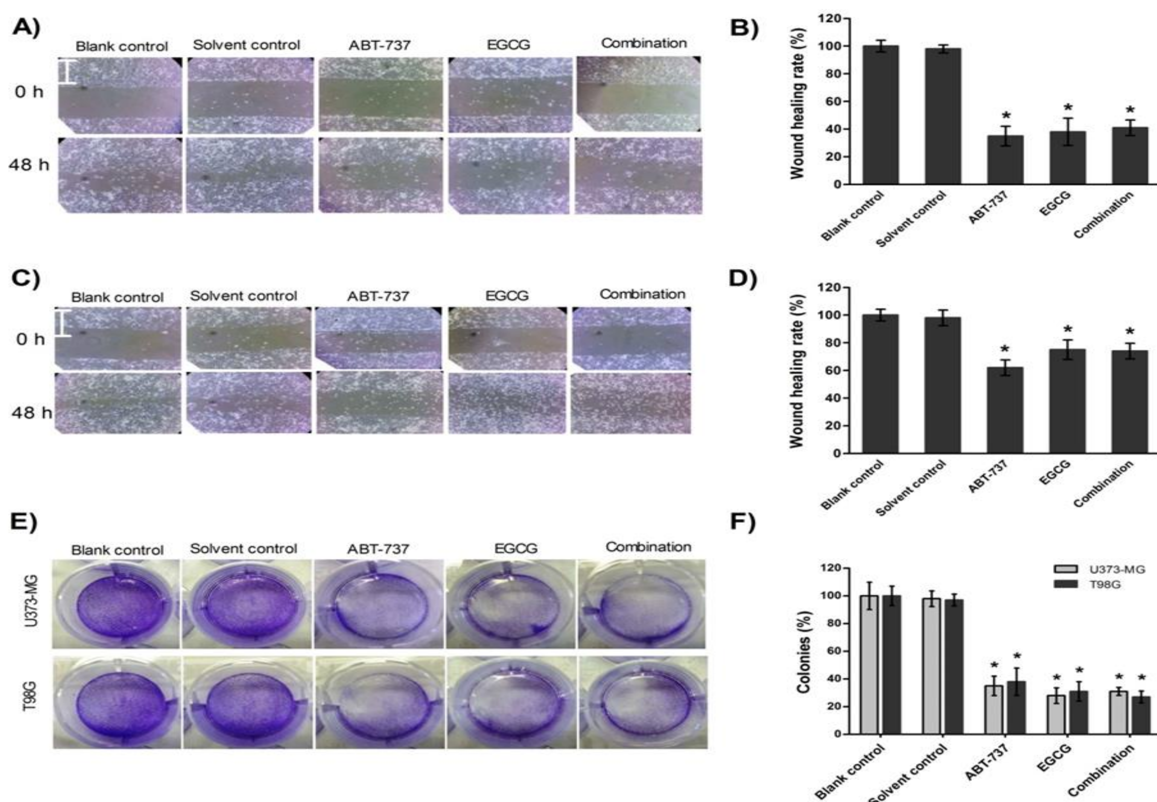

Figure 3. Effect of EGCG and ABT-737 on colony formation and cell migration. After treatment, the migration of U373-MG (A and B) and T98G cells (C and D) was assessed by measuring wound closure areas 48 hr after treatments. Scale bars: 500 micro meter. E and F show the Effect of EGCG and ABT-737 on colony formation in glioblastoma cells. The cell colonies were stained with crystal violet and the number of colonies was observed after 48 hr. Results arerepresentative of three independent experiments. *p<0.05 relative to control groups.

EGCG augmented the inhibitory effect of ABT-737 on colony formation

The clonogenic assay demonstrated that treatment with EGCG and ABT-737 significantly suppressed colony formation in both glioblastoma cell lines. This inhibitory effect was most pronounced in the combination group, which showed a marked reduction in colonies compared to the control or single-agent treatments ($p<0.05$, Figure 3). These results indicate a synergistic interaction between EGCG and ABT-737 in inhibiting the clonogenic survival of U373-MG and T98G cell.

EGCG augmented the apoptotic effect of ABT-737 in glioblastoma cells

Glioblastoma cells were examined for apoptotic morphological changes through the application of Hoechst 33342 staining. Results show that the cells treated with EGCG and ABT-737 displayed the presence of apoptotic bodies which contain nuclear fragments. Conversely, the control cells did not exhibit any such bodies, emphasizing the specific influence of EGCG and ABT-737 on cellular apoptosis (Figure 4).

According to the cell death detection ELISA, a 48-hrtreatment with EGCG or ABT-737 alone significantly induced apoptosis in U373-MG cells by 4.21-fold and 4.11-fold, respectively, compared to the blank control ($p<0.05$). The combination of both agents resulted in an even greater apoptotic response, reaching 4.37-fold. The solvent control showed no significant effect, and this trend was consistently observed in the T98G cell line.

The results of ELISA cell death assay show that the IC_{50} dose required for the combination treatment is lower than the IC_{50} dose needed for either compound treated individually. This indicates that the synergistic effect of the two agents exerts a more substantial effect on the induction of

cell apoptosis compared to the effects of each agent alone. These results are in accordance with the results obtained from the MTT assay.

Caspase-3 activity enhanced after treatment with EGCG and ABT-737

To investigate the mechanism of apoptosis, caspase-3 activity was measured. Treatment with either EGCG or ABT-737 alone significantly increased caspase-3 activation. However, the combination of both agents did not result in a further significant increase in activity compared to the individual treatments (Figure 4).

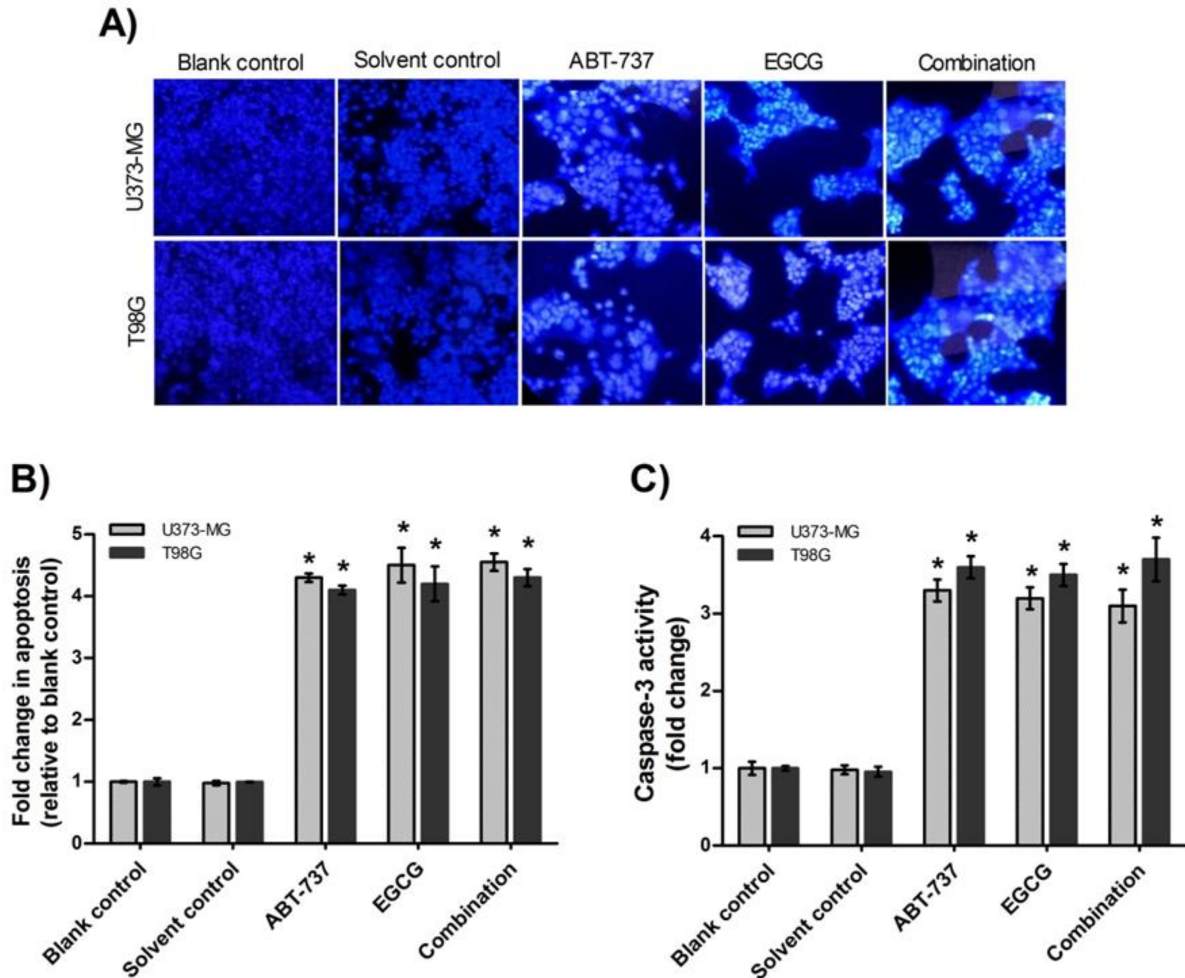

Figure 4. Induction of apoptosis by EGCG and ABT-737 in glioblastoma cells. After 24 hr of treatment, nuclear morphological changes were determined using Hoechst 33342 staining and a fluorescence microscope (A). Fold change in apoptosis was assessed using ELISA cell death assay (B). (C)Caspase-3 activity of glioblastoma cells after 48 hr treatment. ; *$p<0.05$ relative to control groups.

Discussion

Our findings indicate that while both EGCG and ABT-737 individually inhibited cell survival and promoted apoptosis, their combination was significantly more effective. The co-administration produced a synergistic response, marked by a substantial reduction in the IC_{50} value and a greater decrease in cell survival compared to either agent alone. By employing a combination therapy approach, the effectiveness of inhibiting cell growth and inducing cell death has been significantly augmented. This emphasizes the potential advantages of employing these substances together in the treatment of certain conditions. Our results strongly indicate that the impact on cell growth and apoptosis is more pronounced with the

combination treatment due to the lower IC_{50} dose required. This suggests a synergistic effect between the two compounds when administered together, leading to enhanced efficacy in inhibiting cell proliferation and inducing cell death. The association between elevated *Mcl-1* gene expression in cancer cells and resistance to the drug ABT-737 is well-documented in scientific literature. Illustrating this, research by Woo et al. (2017) found that the agent YM155 enhanced the susceptibility of glioma, renal, and lung cancer cells to ABT-737-induced apoptosis through the suppression of *Mcl-1*. Our data are in agreement with the above reports and indicate that EGCG has the ability to increase the susceptibility of glioblastoma cells to ABT-737 and this effect is associated with the suppression of the expression of *Mcl-1* mRNA.

Investigation into the transcriptional effects revealed that ABT-737 induced the upregulation of *Mcl-1* mRNA. In contrast, no significant alteration was observed in the transcriptional output of the *MMP-2* gene. Conversely, EGCG treatment was found to be associated with the reduction the of both *MMP-2* and *Mcl-1* mRNA levels in tumor cells. Furthermore, when we combined EGCG and ABT-737, we observed that EGCG was able to counteract the up-regulation of Mcl-1 mRNA caused by ABT-737. These molecular changes were directly connected to significant functional outcomes, manifesting as inhibited cell proliferation, reduced colony formation, and impaired cell migration. Moreover, our findings indicated that EGCG enhances the sensitivity of glioblastoma cells to ABT-737. Our study aligns with previous research that has explored the relationship between EGCG and its effects on gene expression and cancer cellular processes. Research by Zhang et al. (2008) revealed that EGCG inhibited glioma cell proliferation, an effect characterized by diminished cell viability and an arrest of the cell cycle, specifically a reduction in the S-phase fraction. In a study conducted by Hu et al. (2015), the effects of EGCG, cisplatin, and oxaliplatin on the growth of human colorectal cancer cells (DLD-1 and HT-29) were examined. The results of their investigation revealed that the combination of EGCG with either cisplatin or oxaliplatin displayed a synergistic impact, leading to the inhibition of cell proliferation and the promotion of apoptosis. This suggests that the concurrent use of EGCG and these chemotherapeutic agents could potentially enhance their therapeutic efficacy in treating colorectal cancer. In line with previous research, our data confirms that EGCG exhibits anti-cancer properties by interfering with the proliferation and migration of malignant cells. Additionally, EGCG has been observed to enhance the sensitivity of tumor cells to certain chemotherapy drugs like ABT-737 through the activation of apoptosis. Its ability to sensitize tumor cells to chemotherapeutic agents highlights its potential as an adjuvant in cancer treatment regimens.

The orderly progression of cellular apoptosis is dependent on the activation of two primary signaling pathways: the mitochondrial or intrinsic pathway and the extrinsic pathway (Karami et al. 2014). The intrinsic pathway is triggered by harmful stimuli that originate from within the cell. This leads to the release of cytochrome c and the subsequent activation of caspases-9. In contrast, the extrinsic pathway is initiated by the binding of ligands to death receptors on the cell's surface.

This binding event activates caspase-8. Both pathways ultimately converge at caspase-3, a central component in the apoptotic process. As the central executioner caspase, activated caspase-3 cleaves and activates other caspases, propagating a proteolytic cascade that carries out the ordered events of apoptosis (Karami et al. 2014). The intrinsic pathway of apoptosis is governed by the homeostatic balance between the pro-apoptotic and anti-apoptotic members within the Bcl-2 protein family (Nazmabadi et al. 2024). The

initiation of apoptosis involves the activation of pro-apoptotic effectors Bak and Bax, which oligomerize to induce mitochondrial outer membrane permeabilization (MOMP), resulting in cytochrome c release and caspase activation. Conversely, the anti-apoptotic proteins Bcl-2 and Mcl-1 function to block cell death by binding and inactivating these pro-apoptotic signals (Shahverdi et al. 2020). The efficacy of the BH3-mimetic ABT-737, which potently targets Bcl-2, Bcl-xL, and Bcl-w, is limited by its low affinity for Mcl-1. Empirical evidence links *Mcl-1* upregulation to both primary and acquired resistance against this compound. Consequently, inhibiting *Mcl-1* is considered a promising therapeutic strategy to overcome this resistance and augment ABT-737 sensitivity (Florent et al. 2020; Gunda et al. 2017; Kang et al. 2008; Li et al. 2009; Shen et al. 2019).

The mechanism by which EGCG exerts its effects on cell cycle regulation and apoptosis involves targeting key proteins such as Bcl-2, Bcl-xL, P21, P53, and Cyclin D1. Through the NF-kB, AKT, and PI3K-mTOR signaling pathways, EGCG is able to modulate the expression of these proteins, leading to the suppression of cell growth and the induction of programmed cell death (Almatroodi et al. 2020). EGCG has been found to modulate key proteins involved in both intrinsic and extrinsic pathway of apoptosis. However, the precise mechanism by which EGCG induces apoptosis in cancer cells remains to be fully elucidated (Shirakami and Shimizu 2018). Our study demonstrates that exposure of the glioblastoma cells to EGCG is correlated with the expression of *Mcl-1* mRNA, a protein involved in promoting cell survival. This reduction in *Mcl-1* mRNA levels was strongly associated with the initiation of cellular apoptosis. Additionally, the decrease in *Mcl-1* mRNA expression enhances the sensitivity of glioblastoma cells to the chemotherapeutic agent ABT-737. These findings suggest that EGCG not only induces cell death but also improves the effectiveness of ABT-737 in treating glioblastoma. The modulation of *Mcl-1* mRNA expression plays a crucial role in these effects.

We evaluated the compounds' effects on cell motility and found that both ABT-737 and EGCG independently reduced the rate of migration. Mechanistically, we linked EGCG's anti-migratory effect to its suppression of *MMP-2* gene expression. This result corroborates existing literature, reinforcing that EGCG inhibits migration and metastasis by downregulating *MMP-2,* thereby highlighting its therapeutic potential for controlling cancer progression (Almatroodi et al. 2020).

This study was conducted *in vitro* on glioblastoma cell lines, which does not fully replicate the complexity of tumors in living organisms. The findings require validation in *in vivo* animal models and ultimately in clinical trials. The lack of protein-level assays such as western blotting is a major limitation in our study. This absence prevents any definitive conclusion about the role of Mcl-1 protein in the observed synergy. Furthermore, the precise molecular mechanism, by which EGCG downregulates *Mcl-1*, was not fully elucidated.

In summary, our study underscores the significant therapeutic promise of combining EGCG with ABT-737 for glioblastoma. This combination synergistically lowered the IC_{50} of ABT-737 and potently suppressed colony formation, cell proliferation, and migration while augmenting apoptosis. These effects were mechanistically linked to the downregulation of *MMP-2 and Mcl-1* mRNA by EGCG. Critically, EGCG counteracted the ABT-737-induced upregulation of *Mcl-1*, thereby sensitizing cells to apoptosis. These insights offer a valuable foundation for developing new glioblastoma treatment strategies.

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Conflicts of interest

The authors had no competing interests.

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Ethical Considerations

Ethical approval for this study was granted by the Deputy of Research and Technology at Arak University of Medical Sciences (AUMS) under the reference code [IR.ARAKMU.REC.1401.074].

Code of Ethics

IR.ARAKMU.REC.1401.074

Authors' Contributions

Study concept and design: HK and AA; Acquisition of data: ZSM, HB and HK; Analysis and interpretation of data: ZSM, HK and AA; Drafting of the manuscript: ZSM, HB, HK and AA; Critical revision of the manuscript for important intellectual content: HK and AA; Funding recipients: HK and AA.

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