RESEARCH ARTICLE

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# Combination Therapy with *PIK3R3-siRNA* and EGFR-TKI Erlotinib Synergistically Suppresses Glioblastoma Cell Growth In Vitro

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## Abstract

**Background:** Up-regulation of *PIK3R3* (Phosphoinositide-3-Kinase Regulatory Subunit 3), the regulatory subunit of PI3K is correlated with the drug resistance of the glioblastoma cells. In the present study, the effect of *PIK3R3* siRNA on erlotinib sensitivity of the U373-MG glioblastoma cells was explored. **Methods:** After *PIK3R3* siRNA transfection, the expression of *PIK3R3* mRNA was measured using RT-qPCR. Trypan blue exclusion assay was used to explore the effect of *PIK3R3* siRNA on cell proliferation. The effects of *PIK3R3* siRNA and erlotinib, alone and in combination, on cell survival and apoptosis were measured using MTT assay and ELISA cell death assay, respectively. **Results:** Our data showed that *PIK3R3* siRNA markedly suppressed the expression of *PIK3R3* in a time dependent way, inhibited the proliferation of the U373-MG cells and triggered apoptosis ($p < 0.05$, relative to blank control). Pretreatment with *PIK3R3* siRNA synergistically decreased the cell survival rate and lowered the $IC_{50}$ of erlotinib. Moreover, *PIK3R3* siRNA markedly enhanced the apoptotic effect of erlotinib. **Conclusions:** Our data propose that suppression of *PIK3R3* can effectively triggers apoptosis and enhances the sensitivity of the glioblastoma cells to EGFR-TKI erlotinib. Thus, *PIK3R3* can be a potential therapeutic target in glioblastoma patients.

**Keywords:** Erlotinib- Glioblastoma- *PIK3R3*- PI3K- SiRNA

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## Introduction

Glioblastoma multiform is the most aggressive form of glioma that accounts for over 50% of brain tumors (García-Claver et al., 2013; Alamdari-Palangi et al., 2020a). Common treatments for glioblastoma include surgery, radiotherapy, and chemotherapy with temozolomide, bis-chloroethylnitrosourea and carmustine (Ruano et al., 2008; García-Claver et al., 2013). Despite all medical efforts in recent years, glioma indicates a high resistance to treatment, and patients show a low rate of survival, which underlines the need for effective therapies (Mellinghoff et al., 2005; Ruano et al., 2008; García-Claver et al., 2013; Alamdari-Palangi et al., 2020b). One of the important approaches for treatment that is being investigated is selective targeting of certain molecule and signaling pathways that are involved in the proliferation of glioblastoma cells (Ruano et al., 2008).

The epidermal growth factor receptor (EGFR) is a member of the HER family receptors that is involved in different cell functions including proliferation and differentiation (Amri et al., 2019a; Amri et al., 2019b; Amri et al., 2021). Over-expression of EGFR gene has been extensively observed in different types of human malignant cells, including glioma. In these tumors, EGFR signaling can be restrained at the level of the receptor or through down-stream signaling mediators like PI3K/protein kinase B (PKB)/AKT pathway (Paul et al., 2013; Oprita et al., 2021). The Phosphoinositide 3-kinase (PI3K) pathway is implicated in a wide variety of cancers. It also seems to be associated with the phenotype of glioblastoma cells including survival, invasion, proliferation, cell growth and resistance to treatment (Ruano et al., 2008; Halatsch et al., 2009). PI3K is an intracellular signal transmitter which is activated by several tyrosine kinase receptors and produces a secondary messenger in the membrane (Vredenburgh et al., 2007; Kreisl et al., 2009). Amplification or mutations of *PIK3R3* (Phosphoinositide-3-Kinase Regulatory Subunit 3) gene, which codes for PI3KCA protein, the regulatory subunit of PI3K, have been reported in several solid tumors (Tsao et al., 2005).

Erlotinib is a tyrosine kinase inhibitor (TKI) that exerts an inhibitory effect on EGFR receptor. It is normally used as a secondary line of treatment for brain tumors (Engelman et al., 2006; Bader et al., 2011). The results of several clinical trials performed with tyrosine kinase

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inhibitors indicate specific mechanisms of resistance toward the action of these drugs in glioma cells (Katso et al., 2001; Luo et al., 2003; Zhang et al., 2003; Parsons, 2005). Thus understanding the molecular pathways involved in resistance to treatment with EGFR inhibitors in order to design new combinatorial therapies to help improve response is essential in glioma cells. Studies have shown that the activation of EGFR leads to a higher expression of *PIK3R3* gene in erlotinib-sensitive glioblastoma and ovarian tumors cells, in comparison with that of resistant cells (Shayesteh et al., 1999b; Galanis et al., 2005b). Moreover, it has been demonstrated that the expression of *PIK3R3* gene in erlotinib-sensitive tumor cell lines treated with erlotinib is reduced, while in erlotinib-resistant cell lines the expression remains the same (Soroceanu et al., 2007; Zhang et al., 2007; García-Claver et al., 2013). These results indicate that the over-expression of *PIK3R3* can be one of the major mechanisms of resistance to erlotinib, and thus suggest *PIK3R3* as an interesting potential therapeutic target for improving the response of glioblastoma cells to erlotinib.

Small interfering RNA (siRNA) mediated gene silencing is a powerful tool to suppress the transcriptional expression of a particular gene using a synthetic double-stranded RNA molecule (Karami et al., 2013; Karami et al., 2014). The aim of this study was to investigate the effect of *PIK3R3*-specific siRNA on proliferation, apoptosis, and erlotinib sensitivity of the U-373 MG erlotinib-resistant human glioblastoma cells.

## Materials and Methods

*Cell culture*

The U-373 glioblastoma cells (Pasteur Institute, Tehran, Iran) were cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 15% fetal bovine serum (FBS) (Sigma-Aldrich), 1% sodium pyruvate, 1% antibiotic (100 µg/ml streptomycin, 100 U/ml penicillin) (Sigma-Aldrich) and 2 mM glutamine in a 37°C incubator containing 5% $CO_2$. The cells were passaged with an initial concentration of $1 \times 10^5$ cells/ml and used in the exponential growth phase in all experiments.

*Cell transfection*

*PIK3R3* and negative control (NC) siGENOME siRNAs were obtained from Dharmacon (Lafayette, CO, USA). Prior to transfection, the cells were propagated in RPMI-1640 medium without antibiotics and FBS. Transfection of siRNA (at a final concentration of 100 nM) was done using Lipofectamine™2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. In brief, siRNA and lipofectamine (4 µl/ml of transfection medium) were diluted in Opti-MEM I Reduced Serum Medium (Invitrogen) separately and incubated at room temperature for 5 min. Next, the diluted solutions were gently mixed and incubated for another 15 min at 25°C. The mixtures were then added to the culture medium. After 6 h incubation at 37˚C in a humidified $CO_2$ incubator, the complete growth medium was added to the cells.

*RT-qPCR*

At various time points after transfections, total cellular RNA was extracted by AccuZolTM reagent (Bioneer, Daedeok-gu, Daejeon, Korea) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 µg of purified total RNA by using MMLV reverse transcriptase (Promega, Madison, WI, USA) and oligo-dT primer following the manufacturer's protocols. RT-qPCR was done in the LightCycler 96 System (Roche Diagnostics GmbH, Mannhein, Germany) using SYBR Premix Ex Taq (Takara Bio, Otsu, Shiga, Japan). Each real-time PCR reaction system was: 1 µl of cDNA template, 0.2 µM of each primer, 12 µl of SYBR green reagent, and 6 µl of nuclease-free distilled water. The primer sequences were as follows: forward, 5'- AGTTCCAAATGGAATGAAGGAC-3', reverse, 5'-ACCTCCTCCCTTGAAATATCC-3', for *PIK3R3*, forward, 5'-CTACAATGAGCGTGTG-3', and reverse, 5'- GTCTCAAACATGATCTGGGTC -3', for β–actin,. The initial denaturation step at 95˚C for 10 min was followed by 45 cycles at 95˚C for 20 sec and 60˚C for 1 min. Relative *PIK3R3* expression was evaluated with the $2^{-(\Delta\Delta Ct)}$ method (Shahverdi et al., 2020; Ashofteh et al., 2021), using β-actin as the control gene. Melting curve analysis was performed to check the specificity of the primer sequences and correctness of PCR.

*MTT assay*

Cell toxicity was determined by 3-(4, 5-+Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay. To investigate the effect of siRNAs on the response of U-373 cells to erlotinib, the $2 \times 10^3$ cells/well cells were transfected with 100 nM of either *PIK3R3* siRNA or NC siRNA in 96-well plates for 6 h. Next, different concentration of erlotinib was added to each well. Erlotinib was obtained from Sigma-Aldrich, and a stock solution was prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Subsequently, Twenty-four and forty-eight hours after transfection, 20 µl of 5 mg/ml MTT solution were added to each well. The cells were then incubated at 37°C for 4 hours and the precipitate was resuspended in DMSO (150 µl/well). The absorbance of each well was measured by a microplate reader (Anthos ht III, Anthos Labtec Instruments GmbH, Wals, Austria) at a wavelength of 490 nm with a reference at 650 nm. The survival rate (SR) was calculated using the following equation: SR (%) = (A Treatment /A Control) ×100%. The concentration that produced 50% cytotoxicity ($IC_{50}$) was determined with Prism 6.01 software (GraphPad Software Inc., San Diego, CA, USA).

*Combination effect analysis*

The combination index (CI) analysis based on the Chou-Talalay method was performed to investigate the interaction between *PIK3R3* siRNA and erlotinib (Pirayesh Islamian et al., 2016; Shahverdi et al., 2021). The results of MTT assay was converted to Fraction affected (Fa; range 0-1; where Fa = 1 represents 0% cell survival and Fa = 0 represents 100% cell survival) and analyzed with the CompuSyn software from Combosyn (Paramus, NJ, USA). CI<1, CI=1 and CI>1 indicate synergistic,

additive and antagonistic effects, respectively.

*Cell proliferation assay*

The effect of *PIK3R3* siRNA on the proliferation of U-373 MG cells was determined by the trypan blue exclusion assay. For this purpose, the 1 × $10^5$ cells were transfected with 100 nM of *PIK3R3* specific and NC siRNAs in 6-well culture plates and incubated for 1-5 days. The cells were then harvested and stained with 0.4% trypan blue (Merck KGaA, Darmstadt, Germany). After 2 minutes of incubation, the number of viable (unstained) cells was counted using an inverted microscope (Nikon Instrument Inc., Melville, NY, USA) and a hemocytometer. The percentage of viable cells was calculated as follows: cell viability (%) = (N Test /N blank Control) ×100, where the percentage of viable cell in the control group was considered as 100%.

*Apoptosis ELISA assay*

In order to assess the cell death, mono- and oligonucleosomes released into the cytoplasm of apoptotic cells were measured using a cell death detection ELISA plus kit (Roche Diagnostics GmbH). U-373 cells seeded at a density of 1 × $10^5$ cells/well in 6-well plates were treated with *PIK3R3* siRNA, NC siRNA, the $IC_{50}$ dose of erlotinib, alone or in combination, as described earlier. At 24 and 48 h post-transcription, cells were harvested and cell lysates were collected. Following a 10 min centrifugation of the cell lysates at 200 g, mixtures of 20 µl of the supernatants and 80 µl of immunoreagent (containing anti-histone-biotin and anti-DNA-peroxidase) were transferred to each well of a streptavidin-coated plate. After 2 h incubation at 25°C, the wells were washed with incubation buffer and then, 100 µl of 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was added to each well. Subsequently, ABTS stop solution was added to terminate the reactions. The optical densities were read at 405 nm with an ELISA plate reader (with a reference wavelength of 490 nm).

*Statistical analysis*

The results are presented as mean ± standard deviation (SD) of three experiments. ANOVA and Bonferroni's test were used to determine the statistical significance of differences between groups. A $p < 0.05$ was considered significant. Prism 6.1 software was used to analyze all data (GraphPad Software Inc).

## Results

*The expression of PIK3R3 mRNA was suppressed by siRNA*

The results of RT-qPCR showed that *PIK3R3* siRNA significantly decreased the expression of the *PIK3R3* mRNA in glioblastoma cells in a time-dependent way (compared to the control group, Figure 1A). After 24, 48, and 72 h post-transfection, relative expression levels of *PIK3R3* mRNA were 82.10%, 72.44% and 61.02%, respectively. Notably, treatment with NC siRNA had an insignificant effect on the expression of *PIK3R3* in comparison with the blank control. Amplification curves were sigmoidal and only one peak was observed in the melting curves (Figure 1B, 1C and 1D).

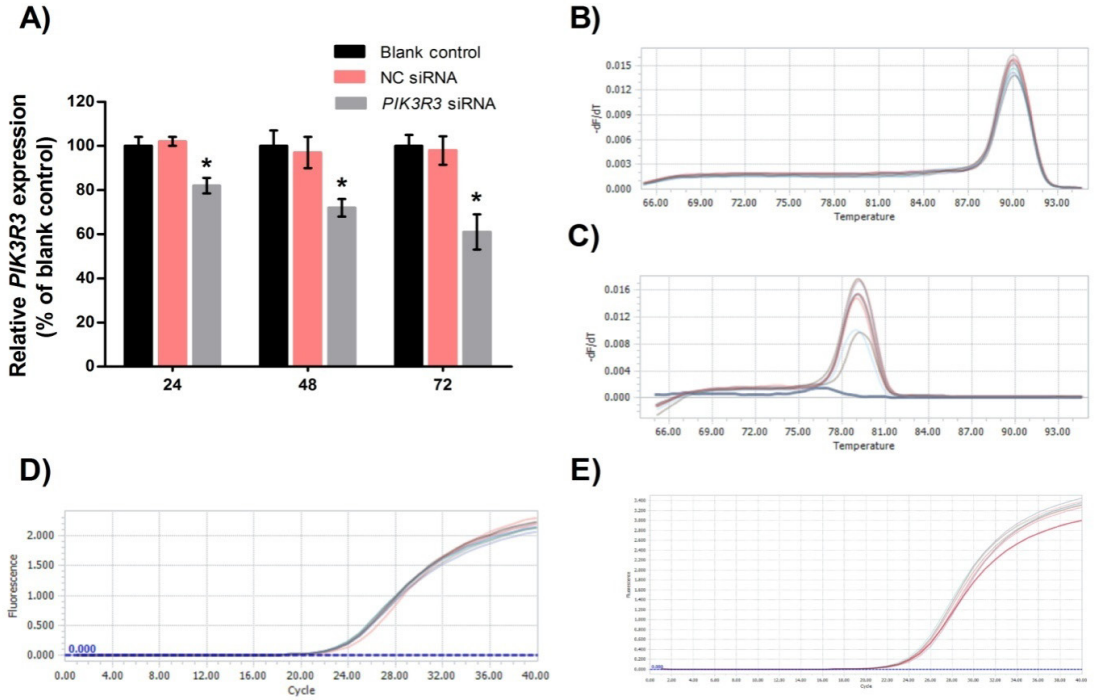


Figure 1. Gene Expression Analysis in U-373 Cells Treated with *PIK3R3* siRNA and NC siRNA . The cells were transfected with PIK3R3 siRNA and NC siRNA for 24, 48 and 72 h. Relative *PIK3R3* mRNA expression was calculated using RT-qPCR and $2^{-(\Delta\Delta Ct)}$ method (A). Figure 1B and 1C show the melting curves, and Figure 1D and 1E show the proliferation curves of β-actin and PIK3R3 genes, respectively. The results are showed as mean ± SD of the results of three experiments. *p<0.05 versus corresponding blank control or NC siRNA transfected cells.

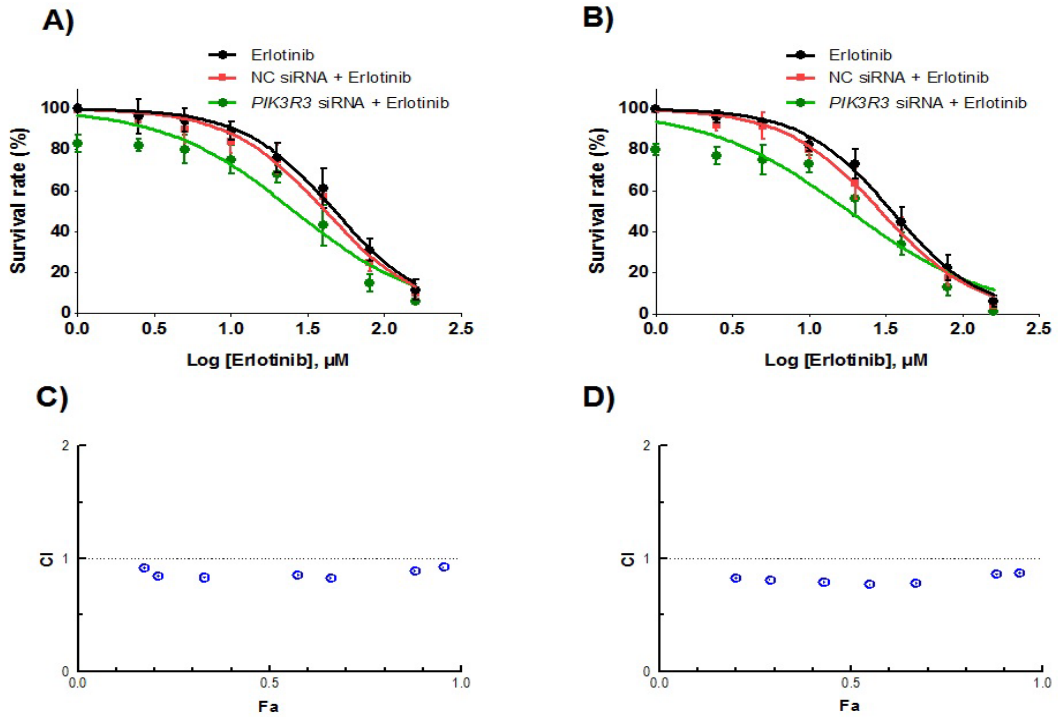


Figure 2. The Effect of *PIK3R3* siRNA on Sensitivity of the U-373 Cells to Erlotinib. Cells were treated with PIK3R3 siRNA and different concentrations of erlotinib for 24 h (A and C) and 48 h (B and D). Next, the cell survival was measured using MTT assay. Cell survival curves (A and B) were plotted using Prism software. The results are presented as mean ± SD (n=3). Data from three independent experiments were used to plot the CI versus (Fa) (C and D) by the Chou and Talalay method and CalcuSyn software. Dashed lines represent CI=1.

Table 1. $IC_{50}$ Values of the Erlotinib in Glioblastoma Cells before and after Transfection of siRNAs.

| Treatment | $IC_{50}$ (μM) | |
|---|---|---|
| | 24 h | 48 h |
| Erlotinib | 44.48 ± 2.35 | 31.84 ± 1.09 |
| NC siRNA and erlotinib | 41.29 ± 1.22# | 28.05 ± 1.67# |
| *PIK3R3* siRNA and erlotinib | 25.86 ± 1.33* | 18.38 ± 0.84* |

$IC_{50}$ values were calculated by sigmoidal dose-response model using Prism software. Data expressed as the mean ± SD of three independent experiments. *p<0.05 versus corresponding erlotinib; #p > 0.05 relative to the corresponding erlotinib.

*PIK3R3 siRNA synergistically enhanced the cytotoxic effect of erlotinib*

A combination treatment of erlotinib and *PIK3R3* siRNA on U-373 cells was investigated, to determine whether decreased *PIK3R3* could enhance the sensitivity of the glioblastoma cells to erlotinib. The results of the MTT assay showed monotherapy with erlotinib induced cytotoxicity in a dose-dependent way (Figure 2A, 2B). *PIK3R3* siRNA significantly reduced the cell survival rate to 84.61% and 79.12% after 24 and 48 h, respectively (compared with the blank control, p<0.05). Furthermore, erlotinib in combination with *PIK3R3* siRNA further reduced the cell survival rate relative to erlotinib or *PIK3R3* siRNA alone (p<0.05). Moreover, *PIK3R3* siRNA markedly reduced the $IC_{50}$ value of erlotinib from 44.48 μM and 25.86 μM to 31.84 μM and 18.38 μM after 24 and 48 h, respectively (Table 1). Meanwhile, transfection of NC siRNA had a minimal effect on the chemosensitivity of the glioblastoma cells compared with the *PIK3R3* siRNA treated cells (p>0.05).

Combination index analysis showed that combination

Table 2. CI analysis of Erlotinib and *PIK3R3* siRNA Combination in U373-MG Glioma Cells

| Erlotinib concentration (μM) | 24 h | | | 48 h | | |
|---|---|---|---|---|---|---|
| | Fa | CI | Combined effect | Fa | CI | Combined effect |
| 2.5 | 0.175 | 0.91 | S | 0.2 | 0.82 | S |
| 5 | 0.21 | 0.84 | S | 0.29 | 0.81 | S |
| 10 | 0.33 | 0.83 | S | 0.43 | 0.79 | S |
| 20 | 0.575 | 0.85 | S | 0.67 | 0.77 | S |
| 40 | 0.88 | 0.88 | S | 0.88 | 0.85 | S |
| 80 | 0.955 | 0.92 | S | 0.94 | 0.87 | S |
| 160 | 0.96 | 0.82 | S | 0.97 | 0.76 | S |

The combination index (CI) values were measured with Chou-Talalay method and CompuSyn software. Antagonistic, synergistic (S) and additive effects are defined by CI value >1, <1 and =1 or close to 1, respectively.

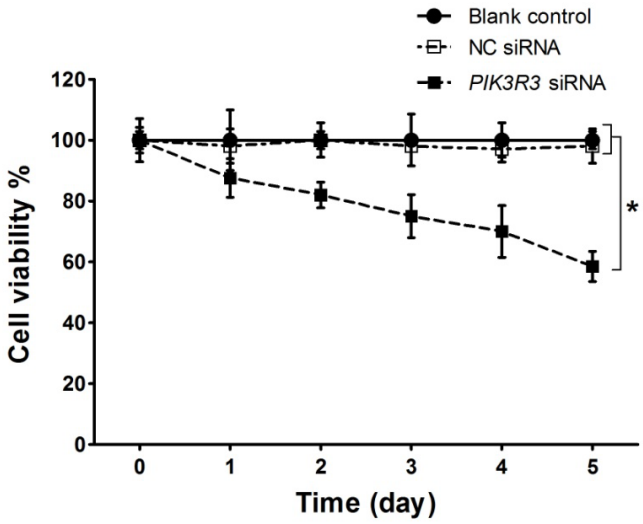


Figure 3. Proliferation Curve of U-373 Cells Treated with *PIK3R3* siRNA. Cell proliferation was determined using trypan blue staining over a period of 5 days. Data are expressed as mean ± SD (n=3). *p<0.05 versus blank control or NC siRNA.

treatment of *PIK3R3* siRNA and erlotinib was synergistic (CI<1) (Figure 2C and 2D). The results showed that the best mean CI value of 24 h were observed at 160 µM erlotinib in combination with *PIK3R3* siRNA (CI=0.82) with Fa level of 0.96. Moreover, strongest synergistic effect of 48 h (CI=0.76, Fa=0.97) was occurred at 160 µM of erlotinib (Table 2).

*Suppression of PIK3R3 expression inhibited the proliferation of U-373 MG cells*

We next sought to examine whether inhibition of *PIK3R3* could inhibit the growth of cancer cells. The U-373 cells were transfected with *PIK3R3* specific siRNA and NC siRNA and then cell viability was assessed using the trypan blue test every 24 h. Results showed that, *PIK3R3* siRNA significantly reduced the proliferation of glioblastomas cells, in a time dependent manner (p<0.05; Figure 3). Beginning at 24 h after transfection, the cell proliferation dropped to 87.10% and then to a further 56.23% on day 5. However, no significant differences in cell proliferation were found between the NC siRNA and blank control groups (p>0.05; Figure 3).

*PIK3R3 siRNA augmented the apoptotic effect of erlotinib in U-373 MG cells*

To determine whether the observed synergistic effect of combination treatment was related to the enhancement of apoptosis, the effects of either erlotinib or *PIK3R3* siRNA, alone or in combination, on cell apoptosis was measured by an ELISA cell death assay Kit. Results demonstrated that compared with the blank control group, *PIK3R3* siRNA significantly enhanced the extent of apoptosis in a time dependent manner (Figure 4). Moreover, 24 and 48 h exposure of the cells to erlotinib augmented the extent of apoptosis by 3.11 and 3.78 fold, respectively (p<0.05). Combination treatment further enhanced apoptosis to

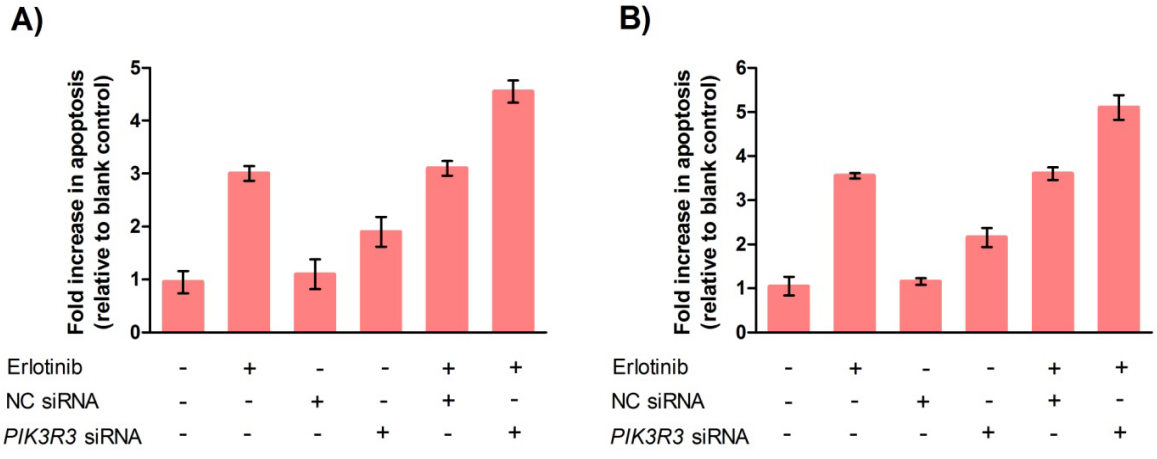


Figure 4. The Effect of Treatments on Apoptosis of Tumor Cells. The U-737 cells were treated with *PIK3R3* siRNA (50 nM), negative control (NC) siRNA (50 nM) and erlotinib ($IC_{50}$ doses of 24 and 48 h), alone and in combination. The apoptosis was quantified using ELISA cell death assay after 24 (A) and 48 h (B). The data are presented mean ± SD (n=3) of three independent experiments. *p<0.05 compared with control.

4.64 and 5.39 fold at the indicated time points ($p<0.05$). No significant alterations in apoptosis were detected for NC siRNA or NC siRNA plus erlotinib groups relative to the blank control group or erlotinib alone, respectively ($p>0.05$). Therefore, these results indicate that the chemosensitization effect of *PIK3R3* siRNA is partially attributed to the induction of apoptosis.

## Discussion

Glioblastoma multiform is the most malignant type of glioma, accounting for more than half of all primary glioma. Although Glioblastoma treatment has progressed in recent years, treatment resistance is high and the overall clinical outcome is still unsatisfactory (Preusser et al., 2008; García-Claver et al., 2013; Alamdari-Palangi et al., 2020a). Thus, developing effective treatments is critical. Erlotinib, a tyrosine kinase inhibitor, is used for treat brain cancers as a second-line medication. Several clinical trials have shown that distinct mechanisms of resistance toward the action of these drugs in glioma cells (Engelman et al., 2006). Knowing the molecular mechanisms responsible for resistance to EGFR inhibitor treatment is essential for developing new combinatorial therapy to enhance response in glioblastoma. In this study, the impact of *PIK3R3* specific siRNA on proliferation, apoptosis, and sensitivity in U-373 MG erlotinib resistant human glioblastoma cells is investigated.

PI3K is an intracellular lipid kinase involved in a wide range of cancer-related pathways. Studies indicated that increased PI3K pathway activity might be due to gene replication or increased expression in cancers also it is associated with tumor cell survival and anti-apoptotic properties (Vivanco and Sawyers, 2002; Galanis et al., 2005a). Over-expression or mutations of the *PIK3R3* gene, the catalytic subunit of PI3K, have been identified in previous studies (Vivanco and Sawyers, 2002; Tsao et al., 2005; Vredenburgh et al., 2007). SiRNAs are small non-coding RNAs that regulate gene expression (Alamdari-Palangi et al., 2020a). Here, we showed that siRNA reduced the expression of *PIK3R3* mRNA, demonstrating that it is one of the main mechanisms of resistance to erlotinib. Moreover, Suppression of *PIK3R3* expression inhibited the proliferation of U-373 cells and increased apoptosis. These findings support *PIK3R3* as a promising potential therapeutic target for increasing glioblastoma cell responsiveness to erlotinib. The results of earlier research revealed that the expression of *PIK3R3* gene in glioblastoma resistant cells has augmented following exposure to erlotinib that may be one of the primary causes of resistance to this drug (Galanis et al., 2005a). MTT analysis in our study indicated that siRNA treatment of glioblastoma cells increased drug sensitivity. In addition, IC50 value of erlotinib was significantly reduced in the presence of *PIK3R3* siRNA. Furthermore, treatment of cells with siRNA led to substantial induction of apoptosis and enhanced cancer cell sensitivity to erlotinib-induced apoptosis. A cell proliferation assay was carried out to ascertain the impact of inhibiting *PIK3R3* gene expression on glioma cell growth. Due to the suppression of this gene by specific siRNA, cell growth was drastically reduced at predetermined intervals. Our findings support previous research by confirming the importance of the *PIK3R3* gene in glioblastoma progression (Shayesteh et al., 1999a; Zhang et al., 2003; Galanis et al., 2005a).

The EGFR is a transmembrane glycoprotein of the ErbB family of RTKs that over-expressed in various types of malignant tumors (Yamaoka et al., 2018). EGFR has been widely implicated in malignant glioma. Approximately 20-50 % of glioblastomas are frequently show high EGFR gene expression and amplification (Raizer et al., 2010). This causes the activation of many signaling pathways including PI3K/Akt and Ras/Raf/MAPK (Li et al., 2013; Sigismund et al., 2018; Singh and Jadhav, 2018; Jamal et al., 2019). Tumor cell proliferation, invasion, and survival are increased when the PI3K pathway is activated. The studies have shown that glioma cells may have specific mechanisms of resistance to anticancer activity. Further analysis of gene expression in susceptible and resistant cell lines prior to erlotinib therapy reveals that genes involved with the PI3K/AKT pathway are over-expressed in the susceptible cell lines. However, over-expression of these genes occurs in the resistant cell line after treatment (Galanis et al., 2005a). A detailed study of PIK3 pathway genes in glioblastoma cells showed a significant difference between sensitive and resistant cells following EGFR stimulation. It is noteworthy that The *PIK3R3* gene, which was expressed more in susceptible cells than in resistant cells, was suppressed exclusively in susceptible cells following erlotinib therapy but no reduction in resistant cells was observed. Furthermore, in glioblastoma proliferation, the copy number of the *PIK3R3* gene enhanced, which could play a role in cancer cell survival (Shayesteh et al., 1999a; Galanis et al., 2005a). These findings suggest that the *PIK3R3* might be an acceptable and interesting therapeutic target for erlotinib responsiveness. In this research, we showed that exposing U-373 MG cells to erlotinib significantly reduced cell survival while increasing apoptosis. Transfection of *PIK3R3* specific siRNA significantly reduced *PIK3R3* mRNA expression and synergistically enhanced erlotinib cell toxicity. Additionally, SiRNA in combination with erlotinib increased the rate of apoptosis comparison to monotherapy. Our observations support the idea that via blocking the PIK3 pathway could augment the antitumor effects of EGFR-TKIs such as erlotinib in glioblastoma cells.

The results of previous reports are aligned with the findings of our study. For example, Ruano et al. (2008) found that genes associated with the PI3K survival pathway play an essential role in glioblastoma patients and are considered to be diagnostic biomarkers for glioma carcinogenesis. Our results also reveal that over-expression of *PIK3R3* plays a vital role in glioblastoma chemotherapy resistance, which could be a pioneer therapeutic target for patients. Zhang et al., (2007) showed that enhanced PI3K pathway activity in ovarian cancer may be owing to gene replication or increased *PIK3R3* expression. They also examined the function of *PIK3R3* by specifically suppressing mRNA expression with siRNA and found that inhibition of the *PIK3R3* mRNA expression enhanced apoptosis in vitro.

Like our study, they showed that anti-*PIK3R3* specific siRNA significantly increased apoptosis and enhanced cellular susceptibility to erlotinib-induced apoptosis. These findings suggest that up-regulation of *PIK3R3* is linked to the erlotinib-resistance of the glioblastoma cells, and *PIK3R3* inhibition could dramatically reduces this resistance.

In conclusion, overall, our results demonstrate that the *PIK3R3* gene has a critical role in the growth, survival and drug-resistance of the glioma cells. Treatment with either erlotinib or *PIK3R3* siRNA markedly triggers apoptosis and inhibits the proliferation of the glioblastoma cells. Moreover, combining *PIK3R3* siRNA with erlotinib improves the therapeutic efficacy of the erlotinib. Therefore, *PIK3R3* could be an effective therapeutic target to reverse erlotinib-resistance of the glioblastoma patients.

## Author Contribution Statement

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

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*Ethical approval*

This research was ethically wise approved from Deputy of research and technology, Arak University of Medical Sciences, Arak, Iran [Number 1170].

*Conflict of interest*

The authors have no conflict of interest to declare

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