Targeting Heat Shock Protein 27 (HspB1) in Glioblastoma Cells with the Combination of Resveratrol and Temozolomide

Elif MERTOGLU, Evren ONAY UCAR

Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, TURKEY

ABSTRACT

Glioblastoma multiforme (GBM) is the most prevalent type of primary malignant brain tumor which has high resistance to chemotherapy. Temozolomide (TMZ) is a chemotherapeutic drug used for treating GBM patients. It is well known that the expression levels of heat shock proteins (HSPs) are generally elevated in various cancer types and they are suitable biomarkers for the therapy. Resveratrol (RSV), a natural polyphenolic molecule, is a potent compound to prevent cancer. In the scope of this study, it was considered that the use of RSV with chemotherapeutic drug TMZ might increase the sensitivity of glioblastoma cells via inhibition of Hsp27 and give positive results for drug efficacy. For this purpose, MTT assay for cell viability, Western blot analysis for Hsp27 and protein carbonyl levels, caspase activity assay for apoptosis, Comet assay for DNA damage and spectrofluorometric analysis for ROS levels were performed in glioma and non-cancerous cells. After optimization of the studies, the doses of 50 μM TMZ and/or 5 μM RSV, which is the optimum result for the viability of the cells, was applied for all experiments. It was shown that combined therapy suppressed Hsp27 level and induced apoptosis in U87MG cells. Furthermore, it has shown that there were no negative effects of this combined therapy in HEK-293 cells. These findings showed that the combination of resveratrol and temozolomide provides a feasible strategy to obtain better therapeutic efficacy by avoiding possible toxicity and side effects in healthy cells.

Keywords: Temozolomide, Resveratrol, Glioblastoma, Hsp27, Combined therapy

INTRODUCTION

Cancer is a very important public health problem worldwide. According to World Health Organization (WHO), the estimated number of deaths in 2018 is 9.6 million, and considering the total mortality rate, 1 out of 6 deaths is reported to be caused by cancer. Brain and other nervous system tumors are not among the most common types of cancer in adult women and men, but they are among the most common in both men and women when the mortality rates are considered. Furthermore, brain and other nervous system tumors are common in young individuals.[1] Brain tumors include high-grade glioblastoma multiforme (GBM), IV. grade astrocytoma is considered the most common and aggressive type. GBM has a poor prognosis, and exhibits abnormal cell proliferation and tumor angiogenesis.[2,3] Standard therapies such as surgical resection, radiotherapy and chemotherapy in patients with brain cancer postpone the progression of the disease and provide a survival advantage but death occurs due to recurrence of the disease after a short time.[4] GBM is also associated with poor prognosis due to its ability to infiltrate normal brain parenchyma. Therefore, although many treatment approaches have been tried, no major progress has been made in improving the survival.[5]

doi: 10.4999/uhod.215019

Heat shock response (HSR) is an important cellular defense mechanism against environmental influences such as heat shock, oxidative stress, and toxins. As a result of HSR in stress-exposed cells, the expression of heat shock proteins (HSPs), also known as stress proteins, increases. It is known that some HSPs have been found to be elevated in many cancer types, therefore they have been accepted as important biomarkers. 27-kDa Hsp27 (HspB1) is a member of small HSPs family and its expression has been shown to increase in glioma cells in many studies.[6-8]

Temozolomide (TMZ), an alkylating agent, is a chemotherapy drug used to treat GBM. Although it is widely used in patients with brain tumor, because of the resistance of glioblastoma to this drug, its therapeutic effect is very limited. Recent years, it has thought that O^6-methylguanine DNA methyltransferase (MGMT) and Hsp27 may play role in this resistance. There are different strategies that can be associated with the resistance to anticancer therapy.[9,10] Resveratrol (RSV) (3,5,4′-trihydroxy-trans-stilbene) is a natural polyphenolic compound which has an anticarcinogenic potential. RSV is present in many foods, especially abundant in grapes, peanuts and red wine. Its low toxicity makes it possible to use higher doses without side effects in humans.[11]

In recent years, there have been studies that combined treatment methods using natural compounds with chemical drugs show important results for drug treatments.[12,13] In some studies, it has been shown that synergistic effects of natural compounds with chemical drugs reduce the side effects on healthy cells.[14,15] In this study, it was aimed by using the combination of TMZ and RSV in human glioma cells to enhance the anticancer activity of TMZ, and also to minimize the side effects in healthy cells by reducing the drug doses. For this purpose, cytotoxic effects of TMZ and RSV on human glioma cell line were evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. According to these results, the different doses of drug and natural compound to be applied to the cells were determined and the effects of their combination on the cells were examined through various parameters. Expression level of Hsp27 were determined by Western blot analysis. In addition, caspase-3 activities, protein carbonyl formations, DNA damage levels and intracellular reactive oxygen species (ROS) determined and evaluated. All experimental studies were performed in both U87MG glioma and non-cancerous HEK-293 cells. Consequently, we investigated therapeutic effect of combined therapy to clarify how RSV contributes to the effects of TMZ on tumor cells. The results of this research may contribute to brain cancer treatment.

MATERIALS AND METHODS

Chemicals and Reagents

TMZ and RSV were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). MTT was purchased from Serva (Heidelberg, Germany). SMART™ BCA Protein Assay Kit was purchased from Intron Biotechnology (Seongnam, Korea). Dimethyl sulfoxide (DMSO), ethyl methanesulfonate (EMS), Caspase-3 Colorimetric Activity Assay Kit and OxyBlot™ Protein Oxidation Detection Kit were purchased from Merck Millipore (Darmstadt, Germany). Hsp27 monoclonal antibody and HRP-conjugated goat anti-mouse IgG were purchased from Enzo Life Sciences (Farmingdale, NY, USA). HRP-conjugated glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading control monoclonal antibody, Pierce™ ECL Western Blotting Substrate and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) were purchased from Thermo Fisher Scientific (Kwartsweg, Bleiswijk, Holland). Cell culture reagents were purchased from Gibco (Carlsbad, CA, USA). Other immunological analysis and Comet assay reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture Conditions

U87MG human glioblastoma cells and HEK-293 human embryonic kidney cells were obtained from Istanbul University Cell Culture Collections. U87MG human glioblastoma cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose containing 10% heat-inactivated fetal bovine serum (FBS), 1% antibiotic-antimycotic solution (100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B)

and 1% non-essential amino acids (NEAA). HEK-293 human embryonic kidney cells were maintained in DMEM/F12 Ham supplemented with 10% FBS, and 1% antibiotic-antimycotic solution. The cells were incubated at 37^{0}C in 5% CO_2 incubator. Experiments were performed using these cells from passages 3 to 15.[16]

Cytotoxicity Assay

Cell viabilities were determined by colorimetric analysis method using MTT assay.[17,18] Briefly, U87MG cells were cultured in 96-well plates at the density 0.5×10^5 cells/mL. TMZ and RSV were dissolved in DMSO. TMZ was added to culture medium at final concentrations of 1-2000 μM, RSV was added at a final concentration of 1-500 μM. In the combined therapy group, different concentrations of RSV (5, 7.5, 10, 15 μM) and TMZ (25, 50, 100 μM) were added to the culture medium. After 24, 48, and 72 h incubation at 37^{0}C, media were removed from plates. The cells were incubated with 30 μL MTT (5 mg/mL in phosphate buffered saline [PBS]) for 4 h, and then 150 μL of DMSO was added to dissolve the formazan crystals formed. Absorbance of this solution was measured at 540 nm using a microplate reader (EON, BioTek Instruments Inc.). The same procedure was applied for HEK-293 cells. These cells were cultured in 96-well plates at the density 1×10^5 cells/mL and treatments were performed for only 48 h.

Western Blot Analysis for Hsp27

The levels of Hsp27 expression in the cells were analyzed by Western blotting according to Onay Ucar et al.[18] The cells were harvested through trypsinization and centrifuged at 3000×g for 5 min. The pellets were suspended in lysis buffer [20 mM Tris-HCl (pH 6.8), 0.04% EDTA, 1% Triton X-100, EDTA-free protease inhibitor cocktail (1 tablet/50 mL), 1 mM PMSF], and homogenized. Then the extracts were centrifuged at 20000×g for 20 min at 4^{0}C to remove insoluble materials. SMART™ BCA Protein Assay Kit was used for the protein concentration of samples. Protein samples (50 μg/well) were separated using SDS-PAGE gel electrophoresis and transferred to PVDF membranes using Bio-Rad Trans-Blot® Turbo™ Transfer System. These membranes were blocked for 1 h using 5% non-fat dry milk (in tris buffered saline and tween 20 [TBST]) at room temperature, then incubated overnight with Hsp27 primer antibody (1:1000) at 4^{0}C. Membranes were washed five times with TBST×5 min, and incubated for 2 hours with HRP-conjugated goat anti-mouse IgG (1:5000) at room temperature, after incubation they were washed again five times. Protein bands were visualized using Pierce™ ECL Western Blotting Substrate kit. HRP-conjugated GAPDH loading control monoclonal antibody (1:2000) were used for normalization of data. The level of the band density was evaluated with the ImageLab 6 software (Bio-Rad).

Caspase Activity Assay

The apoptotic effects of agents/compounds were determined using the Caspase-3 Colorimetric Activity Assay Kit, which evaluates the activity of caspase-3 enzyme, according to the manufacturer's instructions. Cells were suspended in lysis buffer, and incubated on ice for 10 min. The cell lysates were centrifuged at 10000×g for 10 min at 4^{0}C. The supernatants were incubated with 1 mM Caspase-3 substrate Ac-DEVD-pNA at 37^{0}C for 2 h. The pNA standards included in the kit were used to generate standard graphics. Enzyme activity of the samples was measured by a microplate reader at 405 nm.

Detection of Intracellular ROS Levels

The level of intracellular ROS was quantified with CM-H_2DCFDA, which is a chloromethyl derivative of H_2DCFDA. For this purpose, agent/compound treatments were performed on cells grown in 96-well black culture plates. After treatment, the media of each experimental group were removed and the cells were washed with PBS. Cells were incubated with 5 μM CM-H_2DCFDA (in "Hank's balanced salt solution", HBSS) for 15 min at 37^{0}C. At the end of the incubation, 100 μL HBSS was added to the cells by removing the solutions. Kinetic measurements were taken by spectrofluorometer (495 nm excitation, 525 nm emission) at 37^{0}C for 2 hours. Spectrofluorometric results were normalized according to cell numbers.

Determination of Protein Carbonyls

Protein carbonyl groups which are markers of protein oxidation in the cell was determined using OxyBlot™ Protein Oxidation Detection Kit. According to the manufacturer's instructions, 15 µg protein taken from the sample was incubated with dinitrophenylhydrazine (DNPH) for 15 minutes at room temperature. For immunoblotting, protein samples were loaded onto SDS-PAGE gels. The gels were electroblotted to PVDF membrane. This membrane was incubated for 1 hour in 5% non-fat skim milk blocking solution and probed for 1 hour with a rabbit anti-DNPH antibody in a 1/150 dilution. After washing, the membrane was incubated for 1 hour with a goat anti-rabbit secondary antibody conjugated to HRP and then washed again with TBST. Protein bands were visualized using Pierce™ ECL Western Blotting Substrate kit. The levels of the band density were evaluated with the ImageLab 6 software.

Comet Assay

The method described by Olive and Banáth[19] was used for Comet analysis (Single cell gel electrophoresis), which is one of the methods to determine cellular DNA damage. This method was performed in the dark and at 4^0C. For this purpose, cells were cultured in 24-well plates and agents/compounds were applied to the cells after 24 hours. 40 mM EMS was applied to the positive control cells for 1 hour at 37^0C causing DNA damage at the end of 48 hours of treatment. The cells were trypsinized and the cell suspensions in PBS ($1.6x10^4$ cells/mL) were mixed with 1.2 mL 1% low melting agarose and spread onto slides pre-coated with a thin layer of 1% agarose. Slides were incubated overnight in lysis buffer (1.2 M NaCl, 100 mM Na_2EDTA, 0.1% sodium lauryl sarcosinate, 0.26 M NaOH, pH> 13) at 4^0C. After, slides were washed three times for 20 min with electrophoresis solution (0.03 M NaOH, 2 mM Na_2EDTA, pH= 12.3). The electrophoresis was performed for 25 min at 4^0C, 0.6 V/cm. Slides were washed with cold distilled water, and stained using propidium iodide (10 μg/mL) for 20 min. Comet tails, which are indicative of the accumulation of DNA damage, were determined on the slides imaged with fluorescence microscope. The density areas of "head part" and "tailing part" were marked using Image J software. 100 individual cells were evaluated for each experiment. The results were calculated and assessed according to this equation: DNA tail%= 100 × (tailing DNA density)/(cell DNA density).

Statistical Analysis

All data were expressed as the mean ± SD of least three independent experiments and $p<0.05$ value was taken as the level of statistical significance. Statistically significant changes were assessed by one-way analysis of variance (ANOVA) followed by Tukey's and Dunnet's posttests for multiple comparisons. Graphs and statistical analyses were carried out using Graphpad Prism 7 software (San Diego, CA, USA).

RESULTS

Combination of TMZ and RSV Enhanced the Cytotoxic Effects in Glioblastoma Cells

To determine the cytotoxic effects of TMZ and/or RSV on the cell viability, MTT analysis were done. For this purpose, the cytotoxic effects of these compounds/agents applied to U87MG cells were evaluated for 24, 48 and 72 hours. Our results showed that different concentrations of TMZ and RSV induced cell death U87MG cells in a dose and time dependent manner. IC_{50} values of 24, 48 and 72 h in U87MG cells were given in Table 1. Besides, the toxic effects of DMSO as used solvent were examined, treated concentrations of DMSO (below 1%) did not affect cell viability.

Table 1. Time-dependent IC_{50} values of temozolomide and resveratrol in U87MG cells

Agent / Compound	IC_{50} (µM)		
	24 h	48 h	72 h
TMZ	1750.0 ± 1.1	988.2 ± 10.1	175.0 ± 1.1
RSV	186.0 ± 1.3	40.7 ± 1.1	13.9 ± 1.1

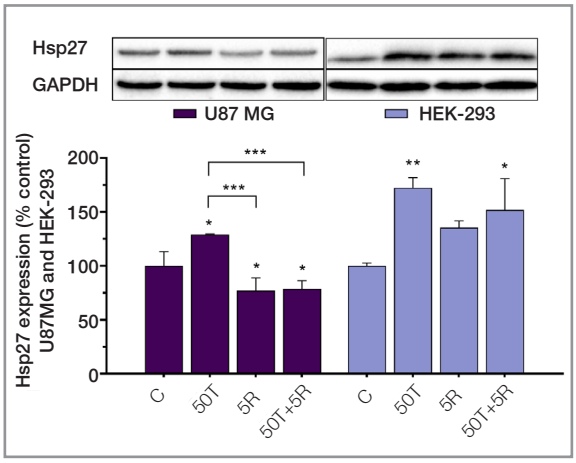

Figure 1. Expression levels of Hsp27 in U87MG and HEK-293 cells treated with 50 μM TMZ and/or 5 μM of TMZ and RSV. All data were normalized to GAPDH (*p< 0.05, **p< 0.01, ***p< 0.001)
(C= Control cells; T= TMZ-treated cells; R= RSV-treated cells).

TMZ and RSV exposure time was determined as 48 hours in U87MG cells by MTT analysis. For this reason, the cytotoxic effects of TMZ and RSV applied to HEK-293 cells were evaluated at only 48th hours. IC_{50} value for TMZ could not be obtained after this process, while IC_{50} value for RSV was determined as 173.78±1.19 μM. Based on these, U87MG cells are more sensitive for TMZ and RSV treatments than HEK-293 cells.

Cytotoxic effects of combined treatments were also evaluated in U87MG cells. TMZ and RSV combinations were applied to the cells in different doses in order to determine the appropriate doses to maintain cell viability. MTT analysis was performed by single or combined different concentrations (25, 50 and 100 μM for TMZ and 5, 7.5, 10 and 15 μM for RSV). As a result, single treatments did not have a statistically significant effect on the cells. Most of the combined treatments caused significant decrease of cells viability except three combined treatments. The combined treatments of 25 μM TMZ/5 μM RSV and 25 μM TMZ/7.5 μM RSV caused 12.2% and 14.6% decrease in cell viability, respectively, and 50 μM TMZ/5 μM RSV treatment resulted in 16.7% decrease in cell viability. In addition, to decide on the appropriate combined concentrations, Hsp27 expression level and caspase-3 activities on the cells were evaluated for optimization. In conclusion, it was determined that the most effective dose was 50 μM TMZ and 5 μM RSV among the doses where viability was not significantly affected. After these optimization studies, it was decided to complete all cellular applications with these treatments. They did not have a significant effect on HEK-293 cell viability.

Combination Treatment Suppressed Expression Level of Hsp27 in Glioblastoma Cells

According to the findings, it was determined that Hsp27 expression was increased with 50 μM TMZ treatment (29.1%) in U87MG cells, but Hsp27 level significantly was reduced by the treatment of 5 μM RSV (22.7%) and the combination of 5 μM RSV and 50 μM TMZ (21.1%) compared to the control. Besides, posttests showed significant differences between the groups (Figure 1). Both RSV and combined treatment suppressed Hsp27 level significantly in U87MG cells compared to the TMZ-treated group. Expression levels of Hsp27 in HEK-293 cells with 50 μM TMZ and/or 5 μM RSV were also examined and there was a statistically significant increase in expression level of Hsp27 in both TMZ- and TMZ-RSV-treated groups in HEK-293 cells (72.4% and 51.9%, respectively), but there was no significant difference in RSV-treated cells.

Combination Treatment Induced Apoptosis of Glioblastoma Cells

The apoptotic effects were determined by evaluating the activity of caspase-3 enzyme in the cells. The results obtained from U87MG and HEK-293 cells are given in Table 2. It was determined that 5 μM RSV was effective in inducing apoptosis in U87MG cells (48.8%) and the combination of 5 μM RSV and 50 μM TMZ showed significant effect according to the control (59.3%). However, it was found that 50 μM TMZ treatment increased caspase-3 activity in HEK-293 cells significantly (57.4%), but either RSV treatment or combination of TMZ and RSV did not make statistically significant differences in these cells compared to control. These results showed that TMZ and RSV combined treatment could get glioma cells more susceptible to apoptosis than normal cells.

Table 2. The results of 50 μM TMZ and/or 5 μM RSV on caspase-3 enzyme activity, ROS, protein carbonyl content and DNA damage in U87MG and HEK-293 cells

Analysis (% control)	Cells	C	50T	5R	50T+5R
Caspase activity	U87MG	100.0 ± 0.7	129.8 ± 19.0	149.4 ± 18.9*	159.3 ± 17.7*
	HEK-293	100.0 ± 1.0	157.4 ± 23.9**	111.5 ± 24.7	129.3 ± 9.5
ROS	U87MG	100.0 ± 7.1	126.6 ± 19.0*	119.7 ± 18.0	135.7 ± 23.5**
	HEK-293	100.0 ± 15.2	92.5 ± 22.5	79.9 ± 12.7	67.9 ± 8.3**
Protein carbonyl	U87MG	100.0 ± 5.4	105.2.± 28.4	90.3 ± 14.5	149.6 ± 27.2**
	HEK-293	100.0 ± 8.8	112.7 ± 14.4	75.3 ± 20.2	101.1 ± 22.0

**p< 0.05, **p< 0.01; C= Control cells; T= TMZ-treated cells; R= RSV-treated cells*

Combination Treatment Enhanced the Intracellular ROS Levels in Glioblastoma Cells

The level of intracellular ROS in the cells was evaluated with CM-H_2DCFDA (Table 2). Each treatment on U87MG cells increased percentage of ROS compared to the control group, but both TMZ and TMZ-RSV treatments caused significantly high ROS level in the cells. Besides, treatments on HEK-293 cells decreased ROS formation compared to the control group. Combined therapy of TMZ and RSV significantly increased ROS levels by 35.7% in U87MG cells compared to the control group, while in HEK-293 cells combined therapy significantly reduced ROS formation by 32.1%.

Combination Treatment Increased Protein Carbonyl Levels in Glioblastoma Cells

In this analysis performed to measure protein damage, it was shown that combined therapy significantly increased protein carbonyl in U87MG cells (49.6%) and did not change in HEK-293 cells (Table 2). It was determined that the combination therapy in U87MG cells showed a significant increase not only compared to the control group but also compared to TMZ or RSV. In addition, treatments of TMZ-RSV in HEK-293 cells did not cause a statistically significant increases compared to the control in terms of protein carbonyl formation. As a result of our applications, TMZ-RSV treatment only significantly increased the amount of carbonyl in U87MG cells.

Combination Treatment Generated DNA Damage

After TMZ and/or RSV treatment, Comet assay was performed to evaluate DNA damage and genotoxicity in U87MG and HEK-293 cells. In this analysis, only medium was applied to the negative control (NC) cells, while EMS causing DNA breaks was applied to the positive control (PC) group. As a result of the analysis, it was found that there were no breaks in the NC cells and high rate of breaks occurred in the EMS treated cells. Fluorescence images of the cells were given in Figure 2. It was determined that 5 μM RSV did not cause DNA breaks unlike 50 μM TMZ in both cells. Combination of 50 μM TMZ and 5 μM RSV was found to increase DNA damage in U87MG cells as statistically significant (54.1%). This combination was also induced DNA damage in HEK-293 cells (71.8%). In both cells, DNA breaks in samples were less than positive control, but these breaks show a statistically significant difference compared to the negative control.

DISCUSSION

RSV is a polyphenol-derived compound and is thought to provide protection against stress conditions. Relatively few research on the interaction of natural compounds with drugs has been reported in the literature.[20] TMZ is the most commonly used drug in the treatment of GBM, which is generally encountered in the central nervous system.[21] Although TMZ-based chemotherapy is known to be

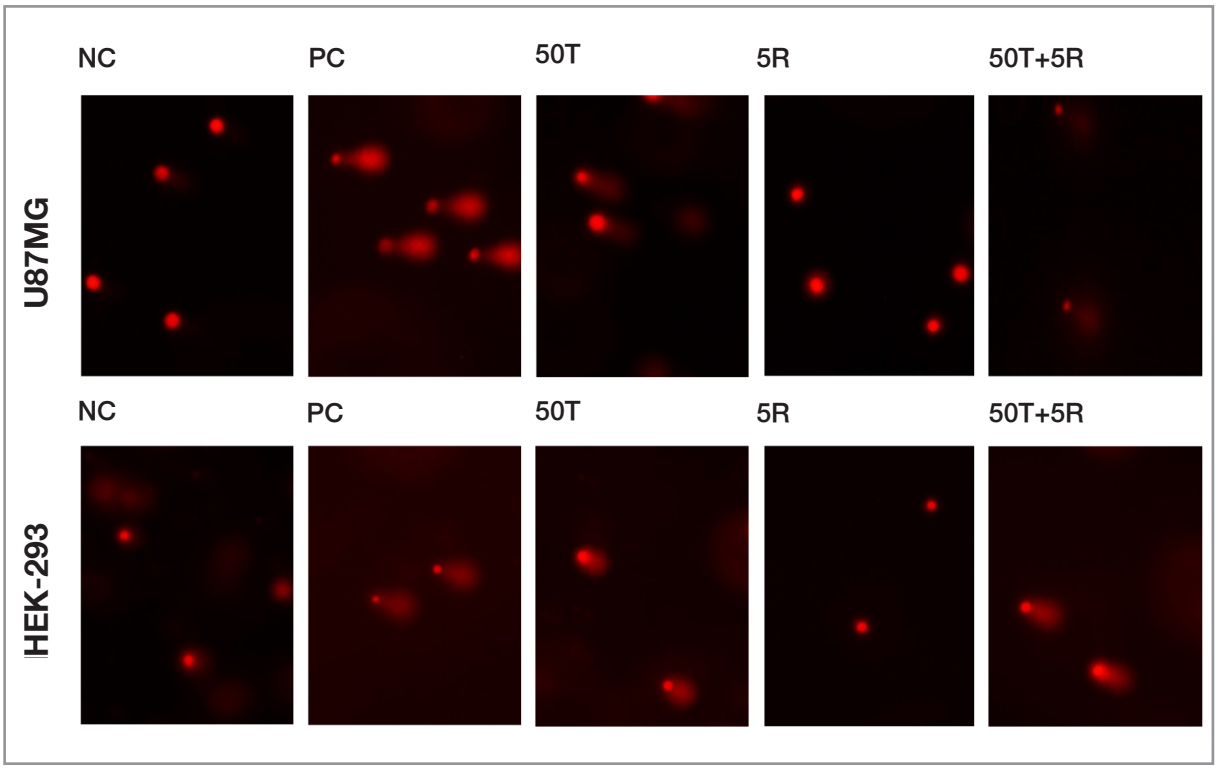

Figure 2. Effects of 50 μM TMZ and/or 5 μM RSV on DNA damage in U87MG and HEK-293 cells. Comet tails showing the presence of DNA damage were determined with a fluorescent microscope.

effective in treatment, not all patients benefit from this treatment in the same way, especially because of the resistance in tumor cells. This is considered to be one of the main causes of failure in the therapy. Directing cancer cells to apoptosis with the treatments have been the most important targets of the researchers lately. Hsp27 is known to be associated with apoptotic signaling pathways. It has a role in reducing oxidative stress and suppressing apoptosis.[22] Studies have shown that Hsp27 inhibition increases this sensitivity to TMZ and decreases drug resistance in glioma cells.[23] Taking all this into consideration, Hsp27 targeted therapies using natural compounds becoming a promising strategy in cancer treatment.[24]

In our research, it was observed that the expression level of Hsp27 in U87MG cells significantly decreased in both 5 μM RSV- and 50 μM TMZ/5 μM RSV-treated groups compared to the control and 50 μM TMZ-treated groups. Moreover, the findings showed that there was no significant difference in Hsp27 expression level of HEK-293 cells in RSV-treated group but Hsp27 expression level increased significantly in TMZ- and TMZ/RSV-treated groups. Castro et al.[10] showed that TMZ treatment increased the expression level of Hsp27. Therefore, this result is in agreement with our findings. Although we previously reported that RSV provided Hsp27 inhibition on glioblastoma cells,[25] Hsp27 expression level was evaluated for the first time in RSV and TMZ-treated group.

The combination of TMZ and RSV are known to induce apoptosis in T98G cells,[26] likewise the combination of quercetin and TMZ induces apoptosis in glioblastoma cells and is more effective than the use of TMZ alone.[23,27] These findings show that induction of apoptosis may be related with Hsp27 suppression. In consistent with above evidences, in this study, combined therapy with TMZ and RSV was found to significantly increase caspase-3 activity and induced apoptosis in U87MG cells. However, the combined therapy did not cause a significant change in caspase-3 activity in HEK-293 cells. The fact that normal cells were not affected by apoptosis shows a positive result.

It is well known that over-production of ROS causes cell death. In a study performed on U87MG cells, intracellular ROS level was found to increase with Hsp27 silencing.[28] In our study, with the combination of TMZ and RSV in U87MG cells compared to the control group, significant increase in intracellular ROS formation was observed. On the contrary, combined therapy resulted in a statistically significant decrease in ROS level in HEK-293 cells. Lin et al. showed that TMZ treatment with RSV in U87MG cells increased in ROS level and caused increased TMZ efficacy by inhibiting autophagy.[29] It is crucial for cell to decide whether the cell goes to autophagy or apoptosis.

Combined therapy significantly increased protein carbonyl in U87MG cells, but it was not significant in HEK-293 cells. In this case, we could say that the suppression of Hsp27 has enhanced the oxidative damage caused by the ROS in glioblastoma cells and these findings suggest that targeting cancer cells with Hsp27 suppression mechanism seems to be good strategy for treatment without harming normal cells. According to the Comet analysis, TMZ- and TMZ/RSV-treated groups produced significant DNA breaks but a relatively low amount of damage was detected in RSV-treated group. Moreover, it was shown that TMZ causes DNA damage in U87MG, Gli36 and DBTRG cells.[10] In another study, it was reported that DNA damage occurred when using 30 μM RSV and 100 μM TMZ in glioma cells, but the source of this damage was defined as TMZ.[21] As the different side of on this subject, we can say that significant amount of DNA damage on cancer cells can occur with lower doses of TMZ and RSV combination, and accordingly it is possible to reduce the side effects of the drug doses. Additionally, it was showed that when the cell detects more DNA damage occurred in the cell than it can repair, then it may activate programmed cell death through activation of caspase-3 and 7 for induction of apoptosis.[30] This also shows us that HEK-293 cells have enough DNA damage that the repair mechanism could manage.

Consequently, it was found that the combination of TMZ and RSV increased the efficacy of treatment in glioma cells. RSV enhanced the therapeutic response of TMZ in glioblastoma cells via Hsp27 inhibition. This combination may provide novel ways for researchers to try new approaches for treatment. RSV is thought to increase in importance in the future. In particular, if the use of RSV in the combination with the drug is clinically supported, it may contribute to the conversion of RSV into a promising agent. Moreover, further investigation may be supported by transcriptomic studies to understand the mechanism of this combination.

Acknowledgement

This study was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project Number: 25989).

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 69: 7-34, 2019.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 100: 57-70, 2000.
3. Iorns E, Lord CJ, Turner N, Ashworth A. Utilizing RNA interference to enhance cancer drug discovery. Nat Rev Drug Discov 6: 556-568, 2007.
4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996, 2005.
5. Louis DN, Holland EC, Cairncross JG. Glioma classification: A molecular reappraisal. Am J Pathol 159: 779-786, 2001.
6. Zhang R, Tremblay TL, McDermid A, Thibault P, Stanimirovic D. Identification of differentially expressed proteins in human glioblastoma cell lines and tumors. Glia 42: 194-208, 2003.
7. Khalil AA. Biomarker discovery: a proteomic approach for brain cancer profiling. Cancer Sci 98: 201-213, 2007.
8. Kampinga HH, Hageman J, Vos MJ, et al. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14: 105-111, 2009.
9. Hirose Y, Berger MS, Pieper RO. p53 effects both the duration of G2/M arrest and the fate of temozolomide-treated human glioblastoma cells. Cancer Res 61: 1957-1963, 2001.
10. Castro NC, Cayado-Gutierrez N, Zoppino FCM, et al. Effects of temozolomide (TMZ) on the expression and interaction of heat shock proteins (HSPs) and DNA repair proteins in human malignant glioma cells. Cell Stress Chaperones 20: 253-265, 2015.
11. Gagliano N, Moscheni C, Torri C, et al. Effect of resveratrol on matrix metalloproteinase-2 (MMP-2) and secreted protein acidic and rich in cysteine (SPARC) on human cultured glioblastoma cells. Biomed Pharmacother 59: 359-364, 2005.

12. Yuan Y, Xue X, Guo RB, Sun, XL, Hu G. Resveratrol enhances the antitumor effects of temozolomide in glioblastoma via ROS-dependent AMPK-TSC-mTOR signaling pathway. CNS Neurosci Ther 18: 536-546, 2012.

13. Gupta SC, Sung B, Prasad S, Webb LJ, Aggarwal BB. Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol Sci 34: 508-517, 2013.

14. Fujiki H, Suganuma M. Green tea: an effective synergist with anticancer drugs for tertiary cancer prevention. Cancer Lett 324: 119-125, 2012.

15. Eid SY, El-Readi MZ, Wink M. Carotenoids reverse multidrug resistance in cancer cells by interfering with ABC-transporters. Phytomedicine 19: 977-987, 2012.

16. Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications. 6th edition. New York, Wiley-Blackwell, 2010: 187-206.

17. Mosmann T. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assay. J Immunol Methods 65: 55-63, 1983.

18. Onay Ucar E, Arda N, Aitken A. An extract from mistletoe, Viscum album L. reduces Hsp27 and 14-3-3 proteins expression and induces apoptosis in C6 rat glioma cells. Genet Mol Res 11: 2801-2813, 2012.

19. Olive P, Banáth JP. The comet assay: a method to measure DNA damage in individual cells, Nat Protoc 1: 23-29, 2006.

20. Ferreira J, Ramosa AA, Almeida T, et al. Drug resistance in glioblastoma and cytotoxicity of seaweed compounds, alone and in combination with anticancer drugs: A mini review. Phytomedicine 48: 84-93, 2018.

21. Filippi-Chiela EC, Thomé MP, Bueno e Silva MM, et al. Resveratrol abrogates the temozolomide-induced G2 arrest leading to mitotic catastrophe and reinforces the temozolomide-induced senescence in glioma cells. BMC Cancer 13: 147, 2013.

22. Vidyasagar A, Wilson NA, Djamali A. Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair 5: 1-7, 2012.

23. Sang D, Li R, Lan Q. Quercetin sensitizes human glioblastoma cells to temozolomide in vitro via inhibition of Hsp27. Acta Pharmacol Sin 35: 832-838, 2014.

24. Mertoglu E, Sengelen A, Kiyga E, Onay UE. Therapeutic drugs and natural products: The effect of suppressing heat shock proteins (Hsp) in brain tumors. In: Heat Shock Proteins in Neuroscience. Asea AAA, Kaur P (eds). Springer, Cham, 2019: 189-208.

25. Onay UE, Sengelen A. Resveratrol and siRNA in combination reduces Hsp27 expression and induces caspase-3 activity in human glioblastoma cells. Cell Stress Chaperones 24: 763-775, 2019.

26. Huang H, Lin H, Zhang X, Li J. Resveratrol reverses temozolomide resistance by downregulation of MGMT in T98G glioblastoma cells by the NF-κB-dependent pathway. Oncol Rep 27: 2050-2056, 2012.

27. Jakubowicz-Gil J, Langner E, Badziul D, et al. Apoptosis induction in human glioblastoma multiforme T98G cells upon temozolomide and quercetin treatment. Tumour Biol 34: 2367-2378, 2013.

28. Ye H, Huang H, Cao F, et al. HSPB1 enhances SIRT2-mediated G6PD activation and promotes glioma cell proliferation. PLoS One 11: e0164285, 2016.

29. Lin CJ, Lee CC, Shih YL, et al. Resveratrol enhances the therapeutic effect of temozolomide against malignant glioma in vitro and in vivo by inhibiting autophagy. Free Radic Biol Med 52: 377-391, 2012.

30. Hassan I, Khan AA, Aman S, et al. Restrained management of copper level enhances the antineoplastic activity of imatinib in vitro and in vivo. Sci Rep 8: 1682, 2018.

Correspondence:

Elif MERTOGLU, Ph.D.

Istanbul Universitesi, Fen Fakultesi

Molekuler Biyoloji ve Genetik Bolumu

34134, Vezneciler-Fatih

ISTANBUL / TURKEY

Tel: (+90-212) 455 57 00 and (+90-530) 313 85 88

e-mail: elif.mertoglu@istanbul.edu.tr

ORCIDs:

Elif Mertoglu 0000-0002-3606-4722

Evren Onay Ucar 0000-0003-1152-4881