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의학박사 학위논문

**Disulfiram, a re-positioned aldehyde
dehydrogenase inhibitor,
enhances radiosensitivity of
human glioblastoma cells *in vitro***

교모세포종 세포주에서

알데히드탈수소효소억제제인 디설피람의

리포지셔닝을 통한 방사선감수성

증강 작용에 대한 *in vitro* 연구

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서울대학교 대학원

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고 현 강

**Disulfiram, a re-positioned aldehyde
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이 논문을 의학박사 학위논문으로 제출함
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**Disulfiram, a re-positioned aldehyde
dehydrogenase inhibitor,
enhances radiosensitivity of
human glioblastoma cells *in vitro***

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A Thesis Submitted to the Department of
Radiation Oncology in Partial Fulfillment of the
Requirement for the Degree of Doctor of
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Seoul National University

by Hyeon Kang Koh
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Abstract

Disulfiram, a re-positioned aldehyde dehydrogenase inhibitor, enhances radiosensitivity of human glioblastoma cells *in vitro*

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Background and purpose: Glioblastoma, the most common brain tumor in adults, has poor prognosis. The purpose of this study was to determine the effect of disulfiram (DSF), an aldehyde dehydrogenase inhibitor, on *in vitro* radiosensitivity of glioblastoma cells with different methylation status of O6-methylguanine-DNA methyltransferase (MGMT) promoter and the underlying mechanism of such effect.

Material and Methods: Five human glioblastoma cell lines (U138MG, T98G, U251MG, U87MG, and U373MG) and one normal human astrocyte (NHA) cell line were cultured and treated with DSF or 6MV x-rays (0, 2, 4, 6, 8 Gy). For combined treatment, cells were treated with DSF before irradiation. Cell survival was evaluated via clonogenic assays. Apoptosis, DNA damage repair, and cell cycle distribution were assayed by Western blot for cleaved caspase-3, γ H2AX staining, and flow cytometry, respectively.

Results: The mean 50% inhibitory concentration of DSF was 71.85 nM for U138MG, 101.91 nM for T98G, 90.78 nM for U251MG, 117.65 nM for U87MG, 80.69 nM for U373MG, and 99.45 nM for NHA. Then, 75 nM for U138MG and 100 nM for the other cell lines were used for subsequent experiments. U138MG and T98G were confirmed with wild type unmethylated promoter (MGMT-wt) because they expressed MGMT protein in Western blot. The other glioblastoma cell lines (U251MG, U87MG, U373MG) were verified as cells with methylated MGMT promoter (MGMT-meth). DSF induced radiosensitization in most of the glioblastoma cells, especially, in the cells with radioresistance as MGMT-wt, but did not in normal NHA cell. The mean sensitizer enhancement ratio at 0.5 of surviving fractions ($SER_{0.5}$) was 1.503 for U138MG, 1.104 for T98G, 1.257 for U251MG, 1.121 for U87MG, 0.945 for U373MG, and 0.998 nM for NHA. The

mean $SER_{0.2}$ was 1.30 for U138MG, 1.091 for T98G, 1.119 for U251MG, 1.10 for U87MG, 0.992 for U373MG, and 0.995 nM for NHA. The mean \pm standard deviation of $SER_{0.5}$ and $SER_{0.2}$ was 1.192 ± 0.236 and 1.121 ± 0.114 for all cells, 1.303 ± 0.273 and 1.196 ± 0.134 for MGMT-wt cells, and 1.148 ± 0.190 and 1.087 ± 0.070 for MGMT-meth cells, respectively. But the mean differences of SER between MGMT-wt cells and MGMT-meth cells were not statistically significant. In the experiments to investigate mechanisms for radiosensitizing effect of DSF, DSF augmented or induced cleavage of caspase-3 in all cells after irradiation. DSF inhibited repair of radiation-induced DNA damage in MGMT-wt cells, but not in cells with MGMT-meth. DSF abrogated radiation-induced G2/M arrest in T98G and U251MG cells.

Conclusion: Radiosensitivity of glioblastoma cells were preferentially enhanced by pre-irradiation DSF treatment compared to normal cell, especially radioresistant cells such as MGMT-wt cells. Induction of apoptosis or inhibition of DNA damage repair may underlie DSF-induced radiosensitization. Clinical benefit of combining DSF with radiotherapy should be investigated in the future.

Keywords: disulfiram, glioblastoma, MGMT, radiosensitivity, DNA damage repair, apoptosis

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INTRODUCTION

Glioblastoma (GBL), the most common brain tumor in adults, has very poor prognosis. Even with the current standard therapy of maximal resection followed by radiotherapy concurrently with temozolomide (TMZ) and TMZ maintenance, the median survival (MS) was 14.6 months in overall patients and less than 24 months in patients with favorable parameters [1,2].

As an anti-mutagenic DNA repair protein, O6-methylguanine-DNA methyltransferase (MGMT) interferes with the anti-cancer effect of TMZ by removing methyl group attached to DNA [3]. Survival of GBL patients depends on the status of MGMT promotor methylation. Those with methylated MGMT promoter (MGMT-meth) have better survival than those with wild type unmethylated MGMT promoter (MGMT-wt) [3,4]. A novel recursive partitioning analysis category has been formulated using MGMT promotor methylation and isocitrate dehydrogenase 1 (IDH1) mutation in addition to traditional demographic parameters for newly diagnosed GBL in TMZ era, emphasizing the status of MGMT promotor methylation as the first partitioning generator [5]. MS was 32.8 months for MGMT-meth and 18.7 months MGMT-wt GBL, respectively ($p < 0.001$).

Since the establishment of standard radiochemotherapy with TMZ around 2006, several attempts have been made to further improve treatment outcomes of GBL, especially GBL with MGMT-wt. Bevacizumab, a monoclonal antibody that binds vascular endothelial growth factor, has failed to provide survival benefit in large phase III studies of AVAglio and RTOG 0825 [6,7]. Although addition of tumor-treating field (TTF) to maintenance TMZ has improved MS from 16.6 months to 19.4 months, the relationship between methylation status of MGMT and treatment outcome remains unclear [8].

Disulfiram (DSF), an aldehyde dehydrogenase (ALDH) inhibitor, has been used for alcoholics since its approval by the United States Food and Drug Administration in 1948 [9]. It produces severe hangover due to accumulation of acetaldehyde after consuming alcohol, and was tolerable without severe toxicities if a person refrains from alcohol consumption [9]. DSF has recently been proposed as a potential repositioned anti-cancer drug [10] or an ALDH targeting agent for cancer stem cells [11]. It induces diverse biological effects such as inactivation of nuclear factor B, accumulation of ubiquitination protein, amplification of reactive oxygen species, inhibition of proteasomes, and suppression of DNA methyltransferases [12]. DSF causes MGMT degradation [13,14]. It has synergistic effects with TMZ on GBL cells [15]. DSF can also potentiate radiation cytotoxicity

in atypical teratoid/rhabdoid tumor (AT/RT) cells [11,16], neuroblastoma cells [17], and pancreatic cancer cells [18]. However, the combined effect of DSF and ionizing radiation on GBL cells has never been reported yet.

Therefore, the objective of the present study was to investigate the *in vitro* radiosensitizing effect of DSF on GBL cells with different status of MGMT promoter methylation and the underlying mechanism of such effect.

MATERIALS AND METHODS

1. Cell culture and drug preparation

Five human GBL cells were used in this study. T98G and U251MG cells were obtained from the American Type Culture Collection. U87MG and U373MG cells were from the Korean Cell Line Bank. U138MG cells and a normal human astrocyte (NHA) cells were kindly provided by Prof. Sun Ha Paek (Seoul National University Cancer Hospital, Seoul, Korea) and Prof. In Ah Kim (Seoul National University Bundang Hospital, Bundang, Korea), respectively. U138MG, U251MG, U87MG, and NHA cells were expanded in Dulbecco's modified Eagle's medium while T98G and U373MG were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics.

DSF was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). It was dissolved in dimethyl sulfoxide (Sigma-Aldrich) to create concentrated stock solutions that were stored at -20°C. A stock solution was diluted in culture medium at the time of use. The 50% inhibitory concentration (IC₅₀) of each cell line was determined using clonogenic assay after exposing cells to increasing concentrations of DSF for 24 hours.

2. Clonogenic assay

Cell survival was measured using clonogenic assay in triplicates as previously reported [19]. Briefly, cells were irradiated with graded doses of 0, 2, 4, 6, and 8 Gy of 6 MV x-ray (Clinac 6EX, Varian Medical Systems, Palo Alto, CA). Cell survival data were fitted to a linear-quadratic (LQ) model [20]. Clonogenic assay was repeated three to four times for each cell line.

3. Western blot for cleaved caspase-3 and MGMT

Western blotting was undertaken as previously reported [21]. Antibodies for cleaved caspase-3 and MGMT were obtained from Cell Signaling Technology (Beverly, MA). Western blot for MGMT expression was repeated three times for all GBL cell lines treated or not treated with DSF for 24 hours. After DSF treatment for 24 hours followed by irradiation with x-ray dose of 6 Gy, cells were subjected to western blot for cleaved caspase-3 at 0, 2, 6, and 24 hours after irradiation. These processes were repeated twice.

4. γ H2AX immunocytochemistry

Immunocytochemistry of γ H2AX as a marker for detecting DNA damage was assayed as previously reported [19]. After exposure to DSF for 24 hours followed by irradiation with x-ray dose of 6 Gy, cells were subjected to γ H2AX

immunocytochemistry analysis at 0, 2, 6, and 24 hours after irradiation. For each treatment condition, numbers of γ H2AX foci in 50 cells were counted. Cells with more than five foci of γ H2AX per nucleus were considered as positive (i.e., containing radiation-induced γ H2AX foci). The process was repeated twice for all cell lines.

5. Flow cytometry

Flow cytometry was performed as previously reported [22]. After exposure to DSF for 24 hours followed by irradiation with x-ray dose of 6 Gy, cells were subjected to flow cytometry analysis at 0, 2, 6, and 24 hours after irradiation using a FACScan (Becton Dickinson, Franklin Lakes, NJ). At least 5×10^5 events were counted. Each procedure was performed twice for all cell lines.

6. Statistical analysis

Kaleidagraph version 3.51 (Synergy Software, Reading, PA) was applied to fit survival data of irradiated cells into LQ model. Mean values between two groups was compared using Student's t-test. All statistical analyses were undertaken using SPSS ver. 18.0 (SPSS, Inc., Chicago, IL).

RESULTS

1. IC₅₀ of DSF

The mean IC₅₀ value \pm standard deviation (SD) was 71.85 ± 0.86 nM for U138MG, 101.91 ± 7.63 nM for T98G, 90.78 ± 2.22 nM for U251 MG, 117.65 ± 45.76 nM for U87MG, 80.69 ± 64.40 nM for U373MG, and 99.45 ± 29.43 nM for NHA. From these results, the value for subsequent experiment was determined as 75 nM for U138MG and 100 nM for others.

2. MGMT expression in GBL cell lines

Western blot results showed that U138MG and T98G cells, but not U251MG, U87MG, or U373MG cells, expressed MGMT (Fig. 1). Thus, U138MG and T98G are MGMT-wt cells while U251MG, U87MG, and U373MG are MGMT-meth cells based on these results, confirming that MGMT methylation is correlated with MGMT protein expression [4]. After treatment of DSF for 24 hours in a concentration of 75 nM for U138MG and 100 nM for others, U138MG and T98G kept the expression of MGMT.

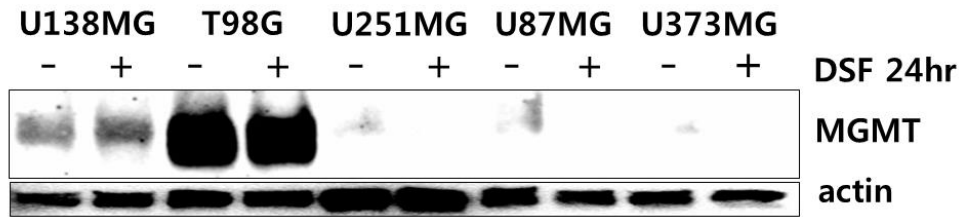


Figure 1. Expression of MGMT (O6-methylguanine-DNA methyltransferase) protein in glioblastoma cells. U138MG and T98G cells expressed MGMT whereas U251MG, U87MG, or U373MG cells did not express MGMT based on Western blots. After disulfiram (DSF) treatment for 24 hours, U138MG and T98G cells maintained expression of MGMT.

3. Enhancement of radiosensitivity with DSF

Radiosensitivity of GBL cells was enhanced by pre-irradiation treatment with DSF except for U373MG. The enhancement was remarkable in MGMT-wt cells (Fig. 2). However, DSF did not affect radiosensitivity of NHA.

Taking the radiation dose that resulted in surviving fractions (SF) of 0.5 and 0.2 as references in cell survival curve fitting after irradiation alone or irradiation plus DSF, sensitizer enhancement ratios (SERs) were calculated (Fig. 2). The mean $SER_{0.5}$ value \pm SD was 1.503 ± 0.251 for U138MG, 1.104 ± 0.065 for T98G, 1.257 ± 0.235 for U251 MG, 1.121 ± 0.072 for U87MG, 0.945 ± 0.034 for U373MG, and 0.998 ± 0.070 for NHA. The mean $SER_{0.2}$ value \pm SD was 1.30 ± 0.084 for U138MG, 1.091 ± 0.070 for T98G, 1.119 ± 0.076 for U251 MG, 1.10 ± 0.035 for U87MG, 0.992 ± 0.019 for U373MG, and 0.995 ± 0.029 for NHA. The mean $SER_{0.5}$ and $SER_{0.2}$ were 1.192 ± 0.236 and 1.121 ± 0.114 for all cells, 1.303 ± 0.273 and 1.196 ± 0.134 for MGMT-wt cells, and 1.148 ± 0.190 and 1.087 ± 0.070 for MGMT-meth cells, respectively (Fig. 3A).

For comparison of its own radiosensitivity of each GBL cell line, surviving fractions at 2 Gy (SF_2) for radiation alone were calculated from cell survival curves. The mean SF_2 value \pm SD was 0.884 ± 0.099 for U138MG, 0.787 ± 0.103 for T98G, 0.826 ± 0.024 for U251MG, 0.719 ± 0.041 for U87MG, and $0.710 \pm$

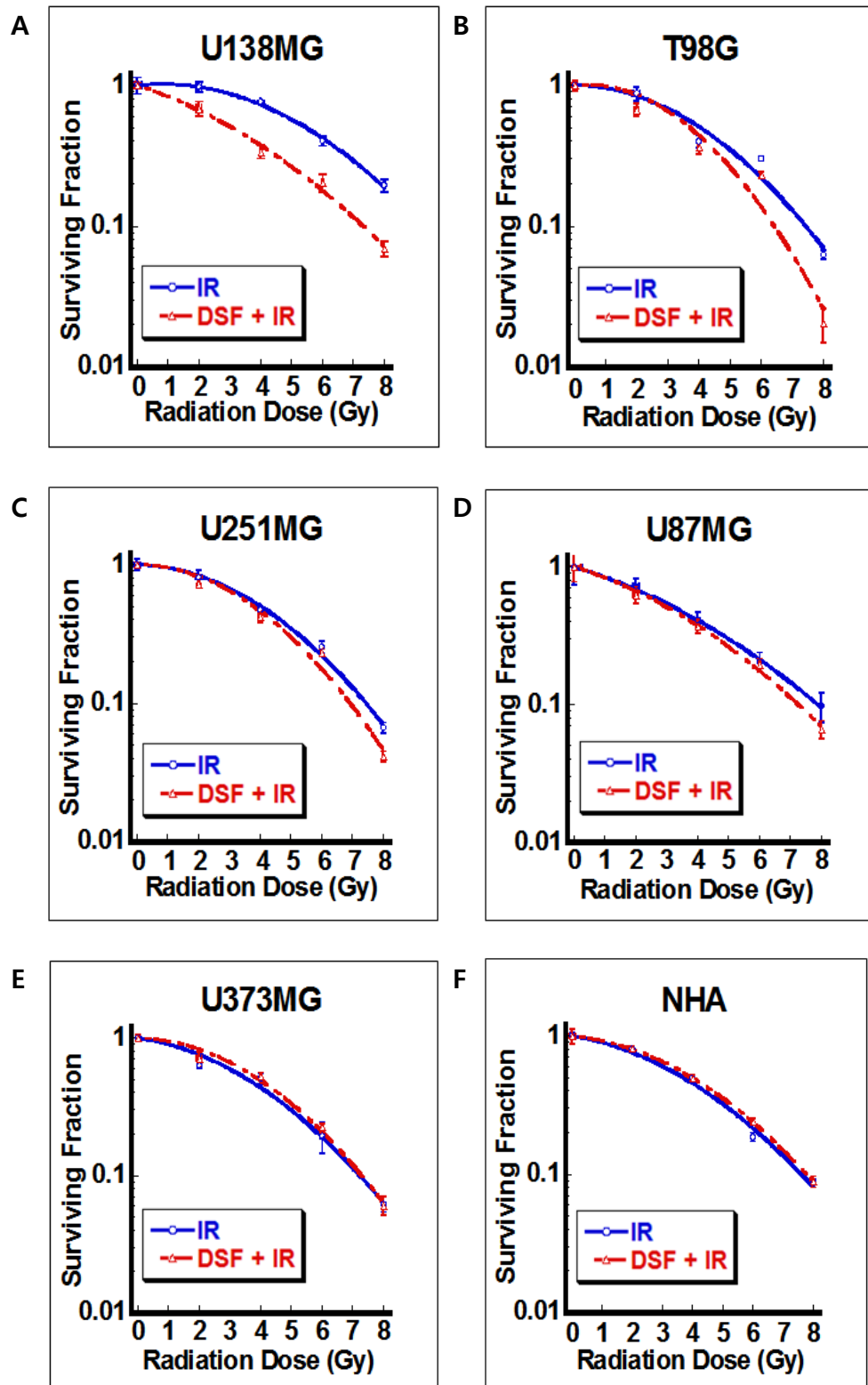


Figure 2. Cell survival curves fitting to a linear-quadratic (LQ) model after irradiation or pre-irradiation disulfiram (DSF) treatment. Radiosensitivity of glioblastoma cells was enhanced by pre-irradiation DSF treatment except for U373MG or normal human astrocyte (NHA). Enhancement was especially pronounced in U138MG and T98G cells with wild type unmethylated MGMT promoter. Each experiment was repeated at least three times with similar results. IR, irradiation; DSF, disulfiram.

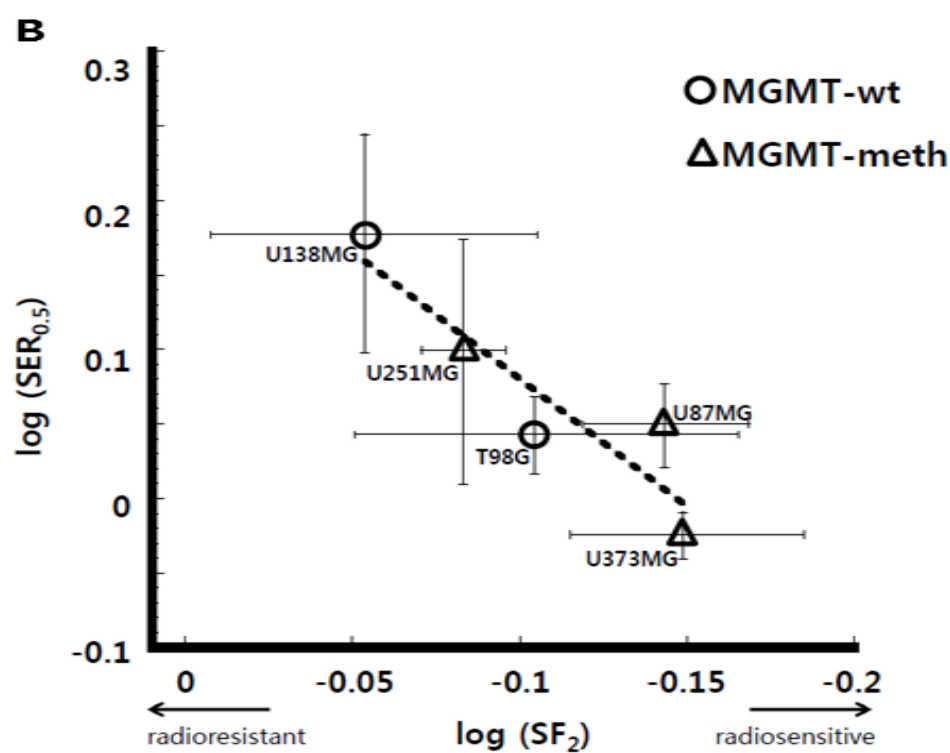
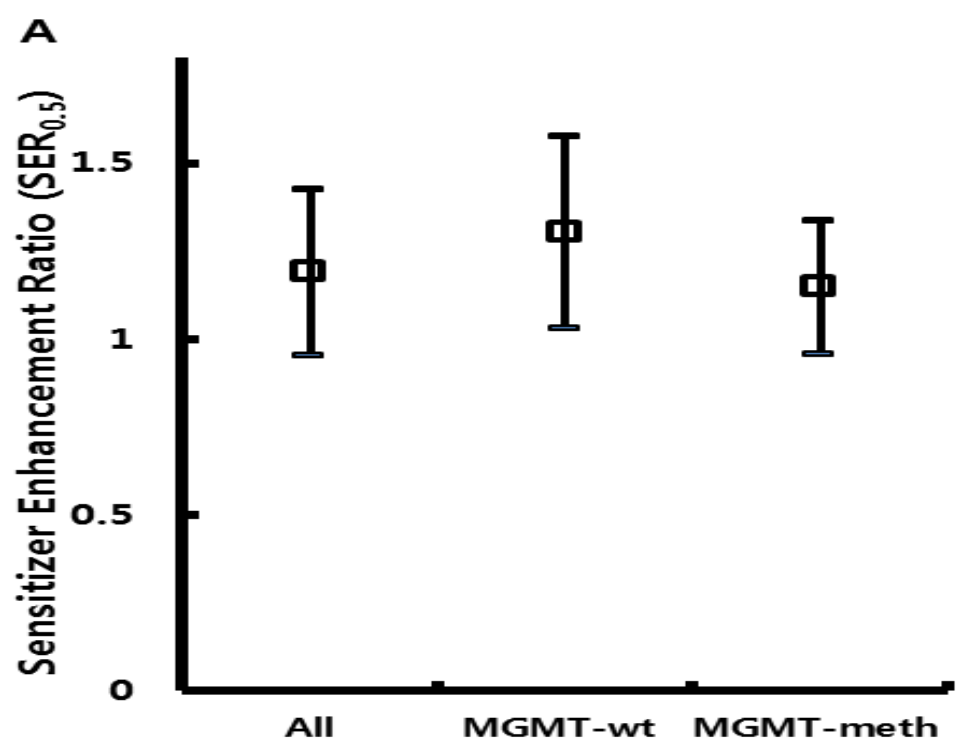


Figure 3. Sensitizer enhancement ratio at 0.5 (SER_{0.5}). (A) Mean SER_{0.5} value \pm standard deviation (SD) in all cells. MGMT-wt cells and MGMT-meth cells are indicated. (B) The relationship between survival fraction at 2 Gy (SF₂) and SER_{0.5} was illustrated in log scale. Error bars indicated SD. There was an inverse relationship between radiosensitivity and the degree of DSF-induced enhancement of radiosensitivity in glioblastoma cells.

0.057 for U373MG. Among these five GBL cell lines, U138MG had the highest SF_2 (i.e., the most radioresistant) while U373MG was the most radiosensitive. When $SER_{0.5}$ was plotted to SF_2 , there was an inverse relationship between radiosensitivity and the degree of DSF-induced enhancement of radiosensitivity in GBL cells (Fig. 3B).

4. Expression of cleaved caspase-3

In T98G and U251MG cells, cleaved caspase-3 was expressed after DSF treatment alone or at 2 or 6 to 24 hours after irradiation alone. Pre-irradiation DSF strongly augmented its expression after irradiation (Fig. 4).

In U138MG, U87MG, or U373MG cells, cleaved caspase-3 was not expressed after DSF treatment alone or up to 24 hours after irradiation alone. However, it was induced to express by pre-irradiation DSF treatment at 6 and 24 hours after irradiation.

5. γ H2AX assay

In MGMT-wt cells (U138MG and T98G), γ H2AX foci existed significantly longer by pre-irradiation DSF treatment (Fig. 5A). The proportion of γ H2AX positive cells after irradiation alone was rapidly and continuously decreased up to 24 hours in U138MG and

T98G cells. However, it was not decreased till 24 hours in U138MG cells or rather gradually decreased in T98G cells with pre-irradiation DSF. The proportion of γ H2AX positive cells at 24 hours was also significantly higher in both U138MG and T98G cells with pre-irradiation DSF in compared with irradiation alone (Fig. 5B).

6. Cell cycle distribution

The proportion of cells at G2/M phase was increased at 6 hours after irradiation alone in U138MG cells, but decreased to control level at 24 hours. This kinetic pattern was not influenced by pre-irradiation DSF treatment (Fig. 6A). However, in T98G cells treated with pre-irradiation DSF, the proportion of cells at G2/M phase at 24 hours after irradiation was lower in compared with irradiation alone.

In MGMT-meth cells, kinetic patterns of U251MG cells were very similar to those of T98G cells. However, proportions at each time points were approximately twice of those measured in T98G cells (Fig. 6A). In U87MG and U373MG cells, the proportion of cells at G2/M phase was increased from 6 hours after irradiation alone. This pattern was not influenced by pre-irradiation DSF treatment.

The proportion of sub-G1 fraction at 24 hours after irradiation was increased compared with that at 0 hour in all cell lines except U373MG (Fig. 6B). These patterns were not affected by pre-irradiation DSF treatment.

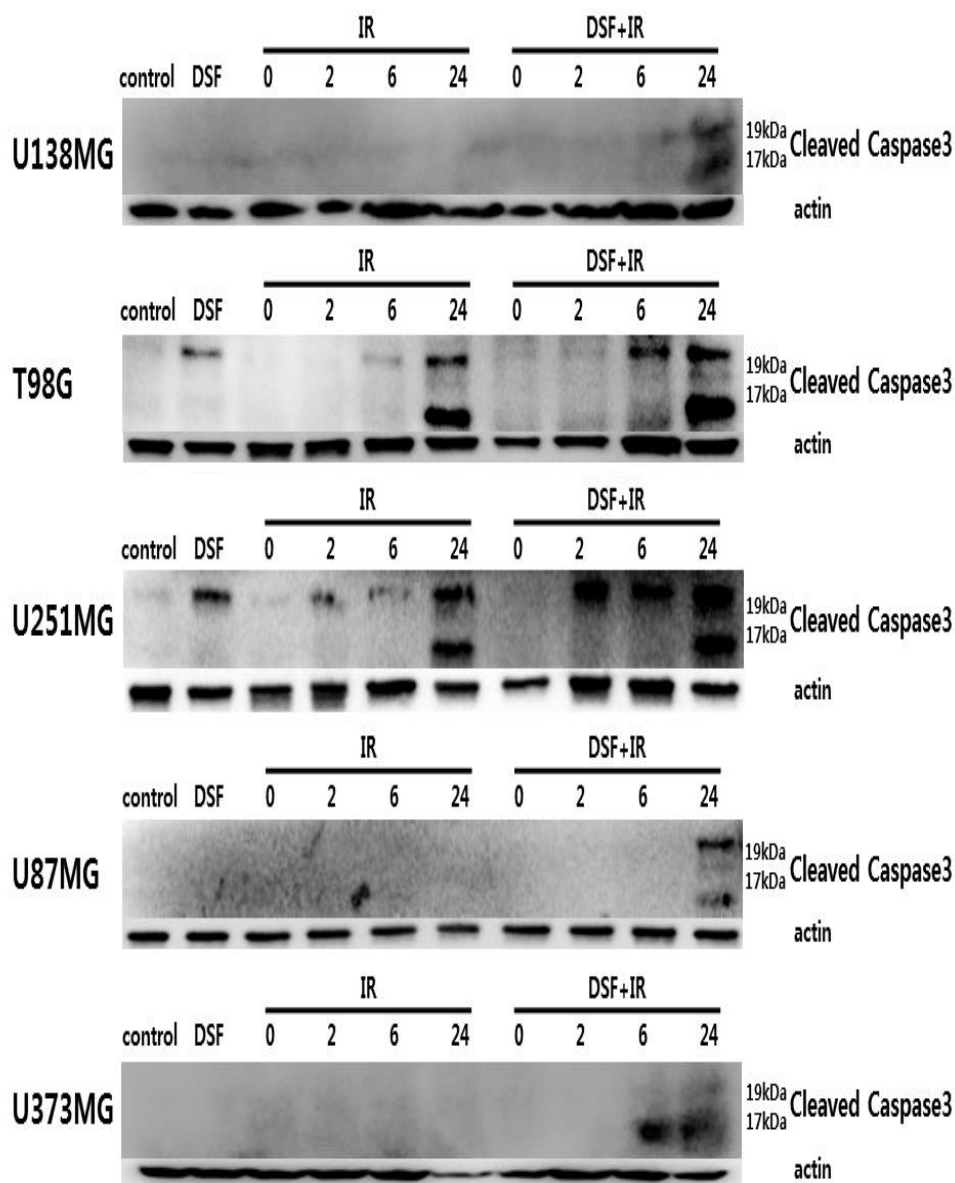


Figure 4. Western blot analysis of cleaved caspase-3 after 0, 2, 6, and 24 hours of irradiation (IR). Pre-irradiation disulfiram (DSF) either induced expression of cleaved caspase-3 in U138MG, U87MG, and U373MG cells or augmented it in T98G and U251MG cells.

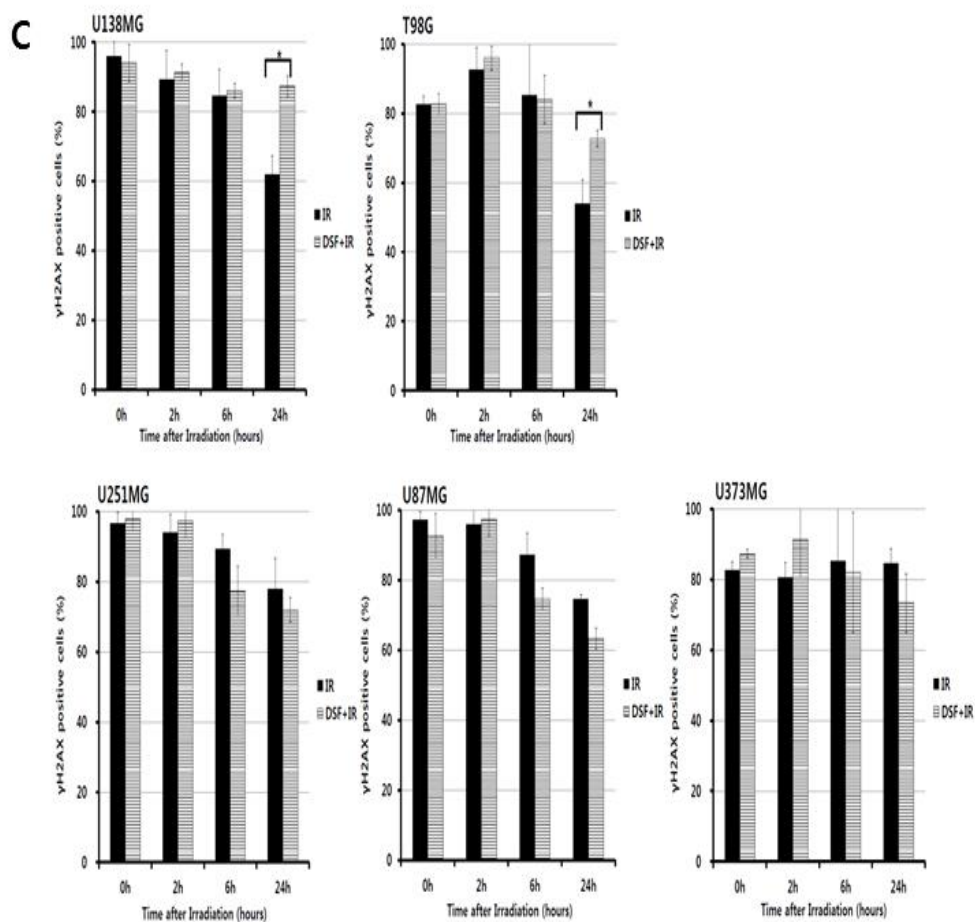
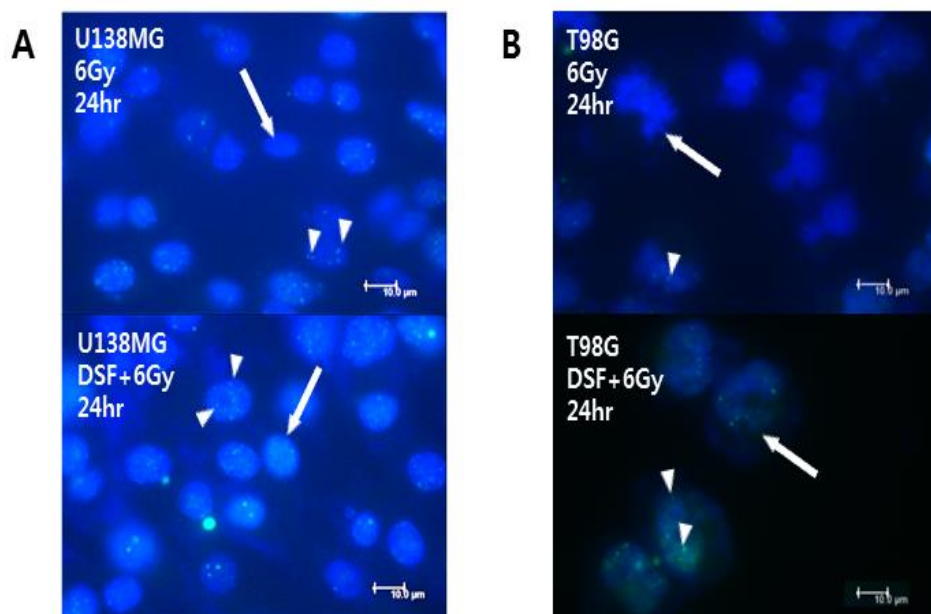


Figure 5. γ H2AX assay. Anti- γ H2AX antibody was applied and then fluorescein isothiocyanate (FITC)-labeled secondary antibody was added (arrowheads). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, arrows). (A, B) γ H2AX foci after irradiation were more prevalent in pre-irradiation disulfiram (DSF) treatment group than those in irradiation (6 Gy) alone group until 24 hours in U138MG and T98G cells with MGMT-wt ($\times 1,000$). (C) The decrease in proportion of γ H2AX positive cells by the time after irradiation was significantly blocked by pre-irradiation DSF in U138MG and T98G cells, but was not in U251MG, U87MG, or U373MG cells.

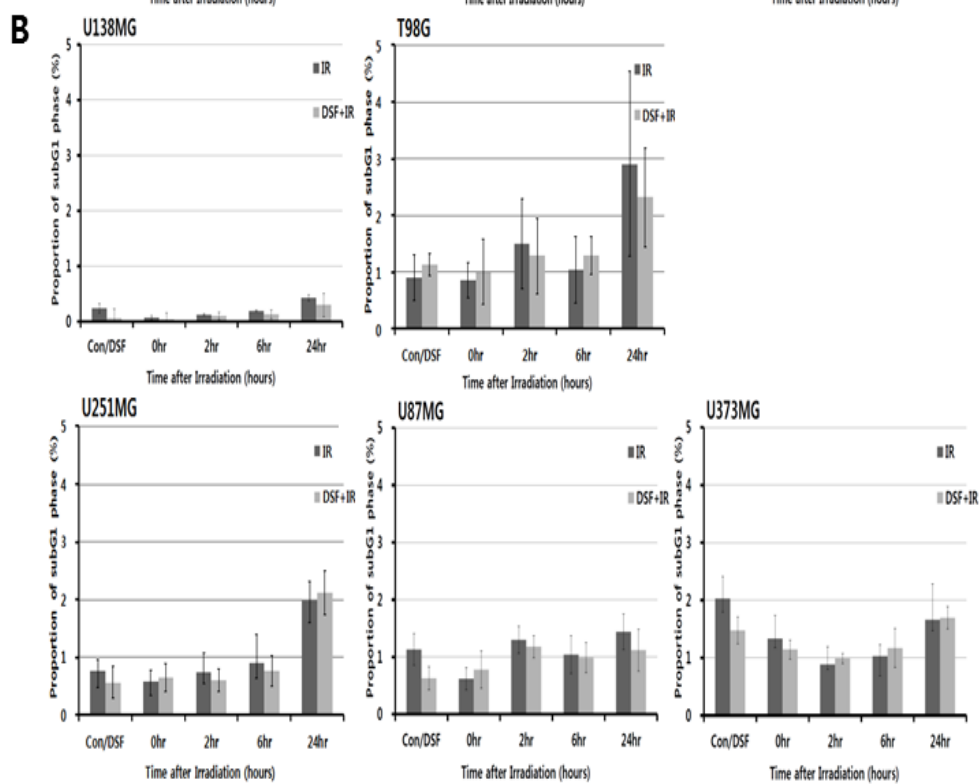
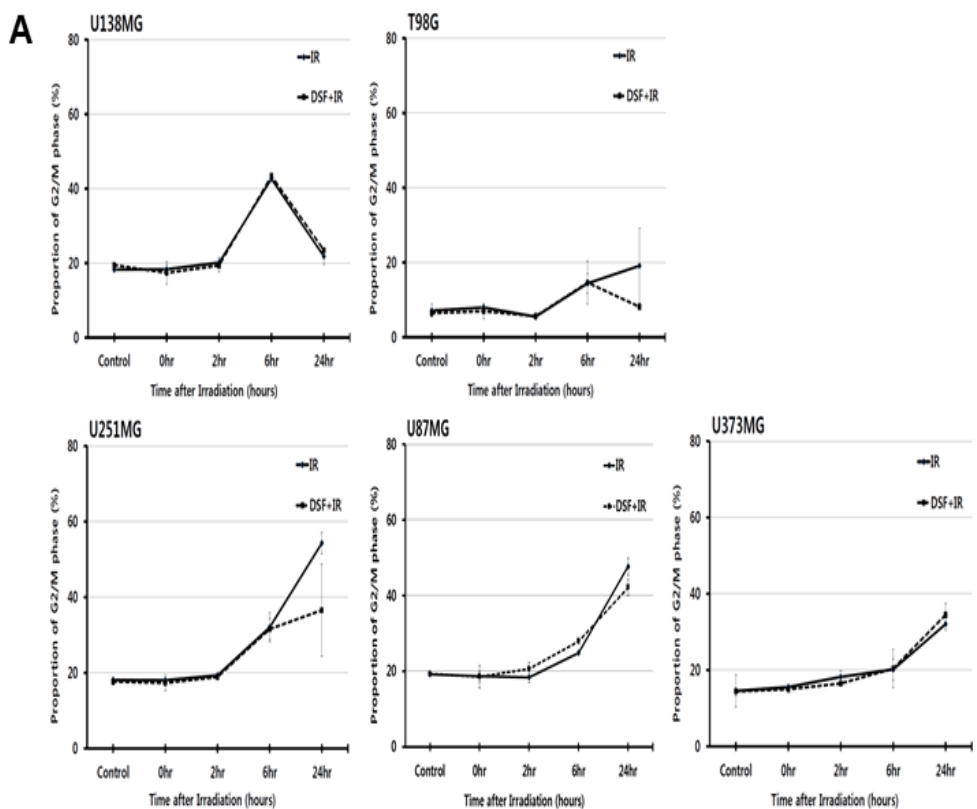


Figure 6. Proportion of cells at G2/M phase and sub-G1 fraction by flowcytometry. (A) Disulfiram (DSF) abrogated radiation-induced G2/M arrest in T98G and U251MG cells, (B) DSF failed to affect radiation-induced change in sub-G1 fraction.

DISCUSSION

This is the first report demonstrating that DSF can induce preferential enhancement of radiosensitivity of GBL cells in compared with normal astrocyte and that DSF-induced radiosensitization is more prominent in cells with radioresistance. These results might have clinical relevance to GBL in general, especially for more resistant GBL with MGMT-wt.

GBLs with MGMT-wt have poor prognosis after standard radiochemotherapy. They have little benefit from TMZ [3]. Therefore, there have been two approaches to improve survival of MGMT-wt GBL. One approach is by developing new treatment strategies and the other is by targeting MGMT itself. Enzastaurin, an orally active protein kinase C and phosphoinositide-3 kinase/Akt inhibitor, has been combined with RT in phase II trial. However, it failed to reach the goal of the study [23]. A phase II study that added cilengitide, an integrin inhibitor, to standard radiochemotherapy with TMZ has reported inconclusive outcomes [24]. There has been no dramatic new treatment for MGMT-wt GBL. On the other hand, MGMT itself has emerged as a treatment target [25]. While inhibitors of MGMT as O6-benzylguanine or O6-[4-bromophenyl] guanine have shown positive results *in vitro* and in animal studies, severe bone marrow toxicities are developed in patients [26].

Synergistic effect of DSF and radiation has been reported in patient-derived AT/RT cells using cell viability assay [11]. It has been further shown that DSF can enhance radiosensitivity of an AT/RT cell line by increasing DNA damage, apoptosis, and autophagy [16]. In a study examining the effect of DSF on sensitivity of neuroblastoma cells to both ionizing radiation and radioisotope, radiosensitization of DSF has been revealed both *in vitro* and *in vivo* [17]. However, the mechanism is not reported [17]. Recently, it has been demonstrated that DSF enhances chemoradiation effect in pancreatic cells by especially targeting cancer stem cells both *in vitro* and *in vivo* [18].

The current study is the first exploration of possible mechanisms of a radiosensitizing effect of DSF in GBL cells. The current study elucidated that pre-irradiation treatment of DSF increased apoptosis in all GBL cell lines. The effect of DSF on cell cycle distribution was inconclusive. It also delayed DNA damage repair, especially in GBL cells with MGMT-wt. Although there have been previous studies that reported DSF alone acts as a direct and potent inhibitor of MGMT or increases DNA damage possibly by posttranslational regulation [13, 14], these effect were not observed in the concentration about IC₅₀ of each cell line in the current study.

DSF has been available for a long time. Its pharmacodynamics and side effects have been well-known. Thus, a phase II trial of DSF as an anti-cancer drug could be accelerated. Costs and time for development of DSF as an anti-cancer drug could be reduced by over 40% [27]. In addition, DSF is inexpensive because the estimated cost of one year for daily treatment with DSF 500 mg is approximately \$550 [27]. Although the economy of cancer treatment should be considered including factors such as expected survival and complications, simply calculated expected cost of other alternative treatments such as bevacizumab or TTF are much more expensive than DFS [28,29]. DSF could be very cost effective from development to dissemination as a cancer drug. Recently, in a phase I study, DSF was used for 12 patients during TMZ maintenance after concurrent chemoradiotherapy. Tolerance dose of DSF was 500 mg daily and the median progression-free survival was 8.8 months, which was favorable compared to historical data [30].

Limitation of the current study was that this was just the first step to generalize the effect of DSF to patients with GBL. Further study is required to confirm the role of DSF as a radiosensitizer in GBL *in vivo* and varied effects according to the methylation status of MGMT promoter of GBL cells.

In summary, DSF has radiosensitizing effect on GBL cells, especially for resistant GBL cells with wild type unmethylated MGMT promotor, possibly due to increased apoptosis and delayed DNA damage repair. Further studies are needed to confirm these findings *in vivo* and/or in clinical studies. Radiotherapy combined with DSF could be a promising strategy for GBL patients, especially for those with wild type unmethylated MGMT promotor and more resistance to radiotherapy alone or radiochemotherapy with TMZ.

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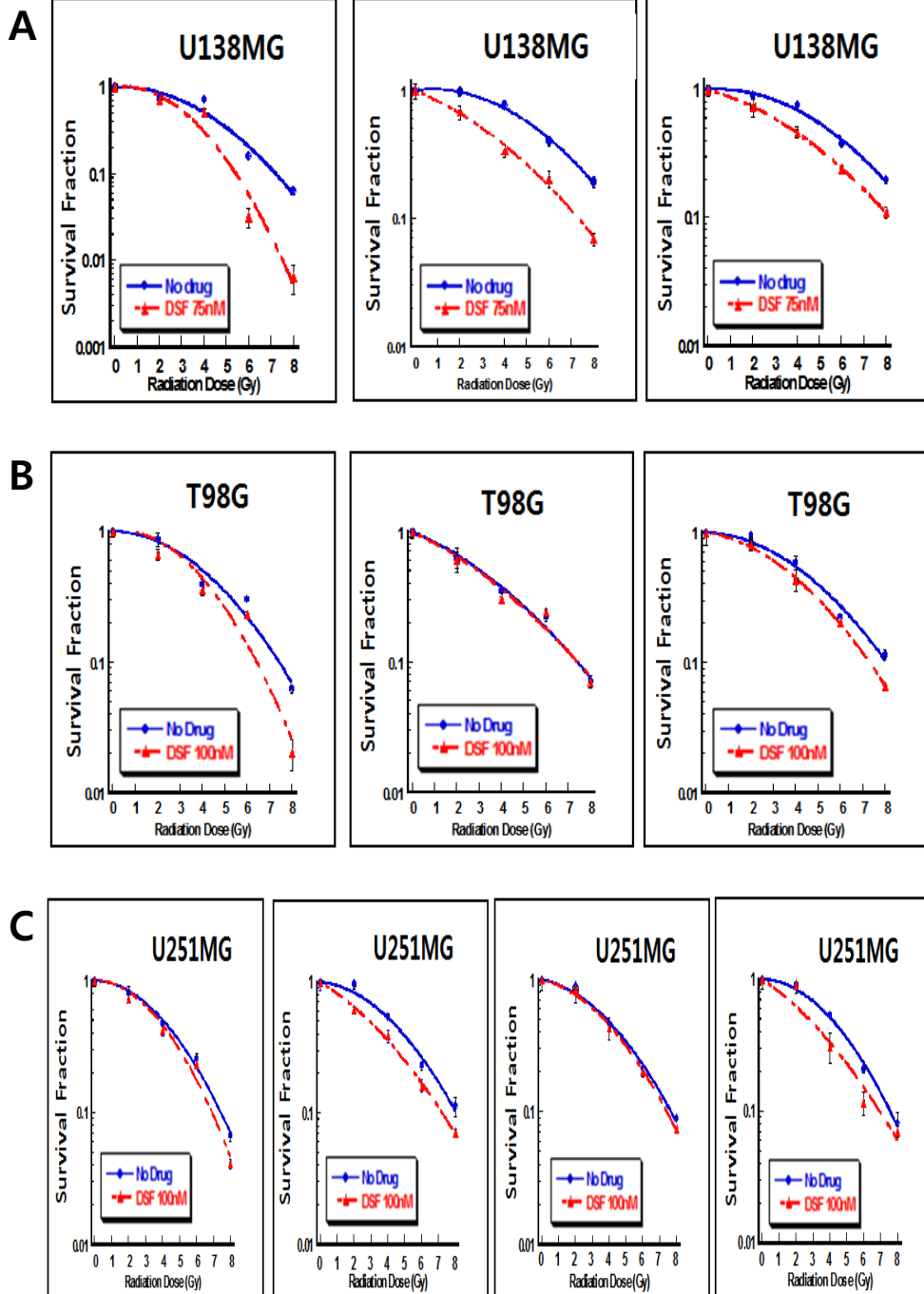
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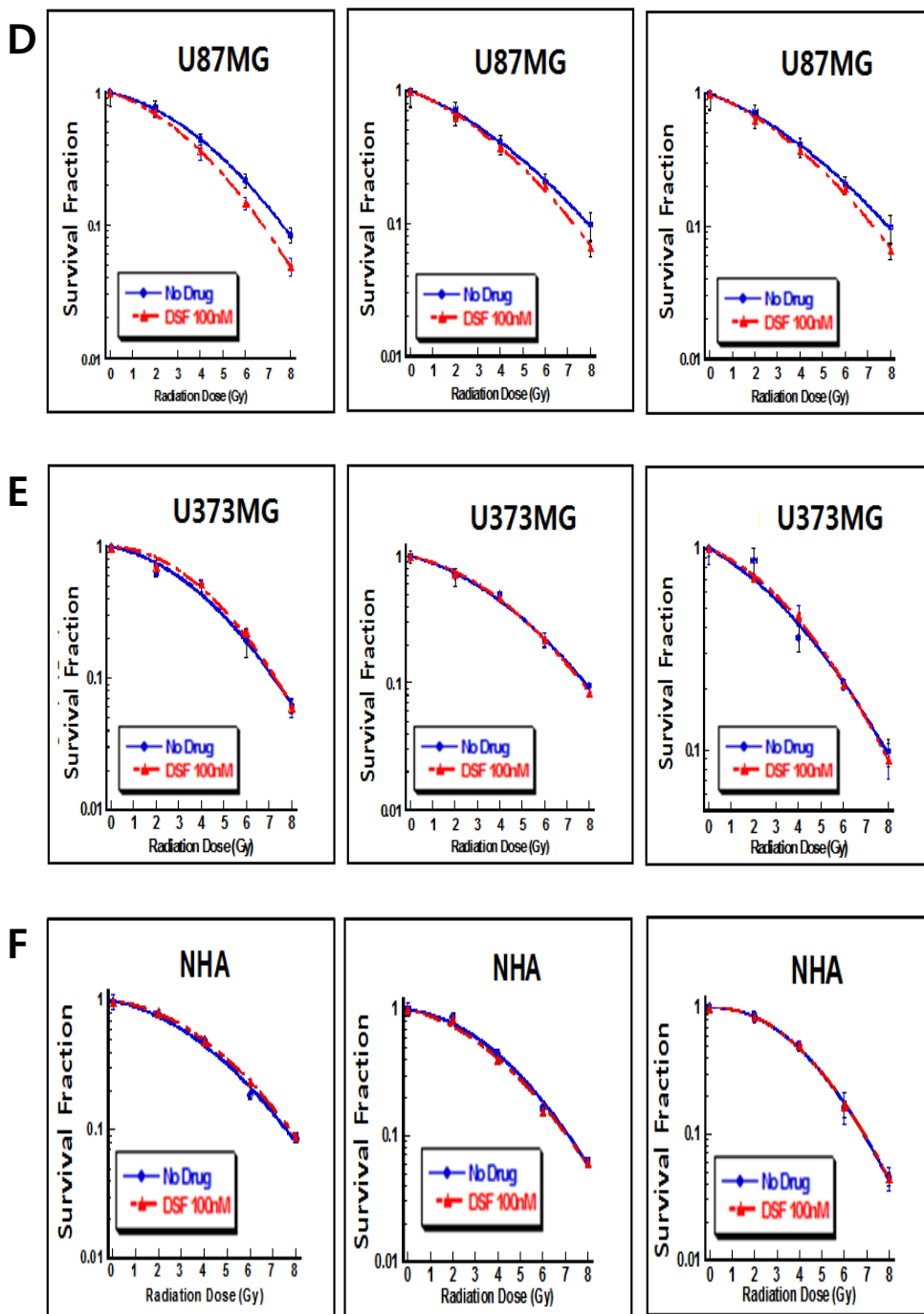
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SUPPLEMENT





Supplement figure 1. Cell survival curves by independent experiments of clonogenic assays. (A) U138MG, (B) T98G, (C) U251MG, (D) U87MG, (E) U373MG, (F) Normal human astrocyte. DSF, disulfiram.

국문초록

교모세포종 세포주에서
알데히드탈수소효소억제제인 디설피람의
리포지셔닝을 통한 방사선감수성
증강 작용에 대한 in vitro 연구

서울대학교 대학원

의학과

방사선종양학 전공

고 현 강

배경 및 목적: 교모세포종은 성인에서 가장 호발하는 뇌종양으로 매우 나쁜 예후를 가진다. 본 연구의 목적은 교모세포종 세포에서 O6-methylguanine-DNA methyltransferase (MGMT) 촉진자의 메틸화 상태에 따라 알데히드탈수소효소억제제인 디설피람이 방사선민감도에

미치는 영향을 알아보고 이와 연관된 기전을 알아보는 것이다

방법: 5종의 교묘세포종 세포주(U138MG, T98G, U251MG, U87MG, and U373MG)와 1종의 정상 별아교세포 세포주 (NHA)를 배양하여 디설피람 또는 6MV X-선 (0, 2, 4, 6, 8 Gy)으로 처리하였다. 병용효과를 보기 위하여 방사선조사 전에 디설피람을 처리하였다. 세포집락형성을 기준으로 세포생존율을 구하였다. cleaved caspase-3 단백질 발현을 Western blot 을 시행하여 세포고사를 측정하였고, γ H2AX 염색으로 DNA 손상 회복을 측정하였으며, 유세포분석법으로 세포주기를 측정하였다.

결과: 디설피람의 평균 50% inhibitory concentration (IC_{50}) 값은 U138MG에서 71.85 nM, T98G에서 101.91 nM, U251MG에서 90.78 nM, U87MG에서 117.65 nM, U373MG에서 80.69 nM, NHA에서 99.45 nM 이었다. 이 결과를 바탕으로 추후의 실험에서 U138MG는 75 nM, 다른 세포주들은 100nM 의 디설피람 농도를 사용하였다. Western blot 결과 U138MG와 T98G가 MGMT 단백질을 발현하여 이 두 세포주가 MGMT 촉진자가 메틸화 되어있지 않은 wild type (MGMT-wt) 세포주임을 확인하였다. 나머지 세포주들(U251MG, U87MG,

U373MG)은 MGMT 촉진자가 메틸화 되어있는 (MGMT-meth) 세포임이 확인되었다. 디설피람이 대부분의 교모세포종 세포주에서 방사선민감도를 증강시켰고 특히, MGMT 촉진자가 메틸화 되어있지 않은 MGMT-wt의 방사선저항성을 가진 세포주들에서 증강효과가 크게 나타났다. 반면, 정상 별아교세포 세포주에서는 증강효과가 나타나지 않았다. 생존분율 0.5 에서의 평균 sensitizer enhancement ratio ($SER_{0.5}$)는 U138MG에서 1.503, T98G에서 1.104, U251MG에서 1.257, U87MG에서 1.121, U373MG에서 0.945, NHA 에서 0.998 이었다. 생존분율 0.2 에서의 평균 sensitizer enhancement ratio ($SER_{0.2}$)는 U138MG에서 1.30, T98G에서 1.091, U251MG에서 1.119, U87MG에서 1.10, U373MG에서 0.992, NHA 에서 0.995 였다. 모든 세포주에서 $SER_{0.5}$ 의 평균 \pm 표준편차는 1.192 ± 0.236 , $SER_{0.2}$ 의 평균 \pm 표준편차는 1.121 ± 0.114 였으며, MGMT-wt 세포들에서는 $SER_{0.5}$ 1.303 ± 0.273 , $SER_{0.2}$ 1.196 ± 0.134 였고, MGMT-meth 세포들에서는 $SER_{0.5}$ 1.148 ± 0.190 , $SER_{0.2}$ 1.087 ± 0.070 이었다. MGMT-wt 과 MGMT-meth 사이의 SER 값의 평균 차이는 통계적으로 유의하지 않았다. 방사선감수성 증강 효과에 대한

기전연구에서 디설피람은 5종의 교모세포종 세포주에서 모두 방사선조사 후의 cleaved caspase-3 단백질 발현을 증강시키거나 유도하였다. 디설피람은 MGMT-wt 세포들에서 방사선조사로 유도된 DNA 손상 회복을 억제하였으나 MGMT 촉진자가 메틸화 되어있는 (MGMT-meth) 세포들에서는 억제하지 못하였다. 디설피람은 T98G 와 U251MG 세포에서 방사선조사로 유도된 G2/M 정지기 세포 분율을 감소 시켰다.

결론: 교모세포종 세포의 방사선민감도는 디설피람을 방사선조사 전에 처리하였을 때 정상 세포와 비교하여 선별적으로 증강되며, 특히, MGMT-wt 세포들과 같은 방사선저항성을 가진 세포들에서 증강되는 경향이 있었다. 세포자멸사의 유도 증가 또는 DNA 손상 회복 억제가 디설피람으로 유도되는 방사선민감도의 내재된 기전으로 판단된다. 이러한 결과로 디설피람과 방사선치료의 병용에 대한 임상적인 이득은 향후 추가적으로 연구 되어야 한다.

주요어: 디설피람, 교모세포종, MGMT, 방사선민감도, DNA 손상 회복, 세포자멸사

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