저작자표시-비영리-변경금지 2.0 대한민국

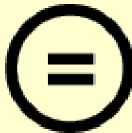
이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.


변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

의학박사 학위논문

Radiosensitization by a novel DNMT Inhibitor, MA 17 and its mechanism *in vitro*

새로운 DNMT 저해제, MA 17의 방사선 감작 메커니즘에 관한 연구

2018년 2월

서울대학교 대학원

의학과 방사선종양학 전공

강 현 철

Abstract

Radiosensitization by a novel DNMT Inhibitor, MA 17 and its mechanism *in vitro*

Hyun-Cheol Kang
Medical Science (Radiation Oncology)
The Graduate School
Seoul National University

Introduction: Epigenetic alteration is known to be an important gene regulatory mechanism. Because the regulation of DNA methyltransferase (DNMT) has been implicated in the regulation of cellular response to radiation, DNMT inhibitors might be considered potential targets for radio-sensitization. We report the evaluation of the *in vitro* radio-sensitizing activities of a novel DNMT inhibitor termed MA-17.

Methods and Materials: A549 (lung cancer) cells were exposed to radiation with or without MA-17. Cell survival curves were obtained via clonogenic assays. We investigated the pathways and the relevant gene ontology associated with any of the

identified differentially expressed genes (DEGs) to seek the underlying mechanism of MA-17 for radio-sensitization. Cell cycle and apoptosis were analyzed via flow cytometry. Expression of γH2AX, a marker of radiation-induced DNA double-strand break (DSB), was examined by immunocytochemistry.

Results: Pretreatment with MA-17 radio-sensitized A549 cells at an IC_{50} of 120uM, where the sensitization enhancement ratio was 1.73 for a survival fraction of 0.5. MA-17 down-regulated DNA homologous recombination and the Fanconi anemia pathway in according to transcriptome analysis, and increased expressions of several genes relevant to the apoptosis pathway were observed. An increased the sub-G1 fraction and prolongation of γH2AX expression of A549 cells were observed in the cells treated with MA-17 prior to radiation as compared with those treated by radiation alone.

Conclusions: MA-17 is the novel DNMT inhibitor, enhanced radio-sensitivity in A549 cells, which is associated with inhibition of repair of DNA DSB and enhanced apoptosis.

………………………………………

keywords: lung cancer, DNMT inhibitor, radiosensitization, epigenetics

Student Number **: 2010-30495**

Contents

Introduction ------------------------------ 1

Material and Methods ----------------------- 3

Results --------------------------------- 7

Discussion ------------------------------ 26

Reference ------------------------------- 30

List of figures

Figure 1. The effects of MA-17 on A549 cell radiosensitivity --8

Figure 2. Pathways relevant to the effect of MA-17 on a radiosensitivity of A549 cells ----------------------12

Figure 3. Influence of DNMT inhibitors on cell cycle phase distributions of A549 cells ------------------------17

Figure 4. Western blot analysis of cleaved caspase-3 --18

Figure 5. Radiation-induced γH2AX foci --------------21

Figure 6. Influence of DNMT inhibitors on radiation-induced γH2AX foci ---------------------------------23

Figure 7. Western blot analysis of FANCA, BRCA1, Rad51C --24

List of tables

Table 1. MA 17-associated pathways for radiosensitivity by Kegg pathway ---------------------------------10

Table 2. List of genes in DEG analysis relevant to the apoptosis of the Kegg pathway -----------------------------14

Introduction

Research on tumor biology has established the important role of epigenetic alterations in the initiation and progression of cancer [1-4]. DNA methylation and alteration of histone code are two principal mechanisms those are involved in epigenetic carcinogenesis and tumor progression. Treatments targeting this 'non-coding' part of biologic process have been tried and the successes observed in hematologic malignancies [5] provoked preclinical and clinical researches for clinical application of epigenetic drugs in solid tumors [6].

It has been suggested that radiotherapy introduces epigenetic alterations [7]. Because epigenetic changes can potentially influence radiation response and following clinical outcome, they may be considered as potential candidate targets of anticancer therapy that modulates this process forward to a positive direction as an enhancer of radiation response, so called radiation sensitizer. A growing number of studies focused on clinical applicability of epigenetic drugs as radiosensitizers [8].

A non-nucleoside inhibitor, psammaplin A (PsA), is an epigenetic modulator found to sensitize human cancer cells to radiation lethality. We have previously shown that PsA induces radiosensitivity in lung cancer cell line [9]. However, it has been demonstrated to be unstable in all biological matrices and to be

too rapidly eliminated in vivo in subsequent pharmacokinetic study [10] to use in vivo studies. As a succeeding step, we synthesized a novel PsA-derived compound which has an improved bioavailability and it was named as 'MA-17'.

In the present study, we tried to evaluate the radio-sensitizing activities of MA-17 in human lung cancer cell line. Up to recent, the molecular pathway that contributes to the underlying mechanism of DNMT inhibitor on enhancing a radiosensitivity of cancer cells is not well understood. While some results suggest possible mechanisms of radiosensitization including alteration of cell cycle, apoptotic, and DNA repair pathway [9,11-13], no studies have shown definitive mechanism in gene expression level. In order to gain insight, an unbiased approach should be employed and identifying differential gene expression method has a potential to be adopted for cancer cells relevant to genetic or epigenetic change following radiation response modifying agent. The aim of this study is to elucidate the mechanisms of the novel synthetic DNMT inhibitor using next generation sequencing (NGS) of RNA and transcriptome analysis.

Materials and Methods

1. Cell culture

As a radio-resistant cell line, A549 cell line (Korean Cell Line Bank, Korea) was chosen. It is a human non-small lung cancer cell line. NHA cell line was selected as a normal cell line (Korean Cell Line Bank, Korea), it is a normal human astrocyte cell line. A549 cells were cultured in RPMI media and NHA cell in DMEM media, both supplemented with 10% fetal bovine serum and 12.5 μg/ml of gentamicin at 37° C in saturated air with 5% CO2.

2. Clonogenic cell survival assays after drug and radiation treatment

Cells were trypsinized and the appropriate numbers of cells were seeded and incubated for 24 hours prior to treatment. Radiation treatment of A549 cell lines was carried out using a linear accelerator (Clinac 2100 C or Clinac 21EX, Varian Medical systems, Palo Alto, CA, USA) with 6 MV x-rays. DNMT inhibitors (PsA, MA-17, 5-aza-2'-deoxycytidine) were administered for 24 hours before radiation treatment. MA-17 has been demonstrated to have a higher bioavailability when compared with PsA: 1.0 ± 0.3 versus 0.16 ± 0.02 hr in the

elimination half-life and 184 ± 49.9 versus 925 ± 570 ml/min/kg in the systemic clearance. After incubation period for 14 to 21 days, the colonies were fixed with methanol and stained with 0.5% crystal violet. Colonies were counted when the number of colonies containing at least 50 cells was determined, and the surviving fraction (SF) was then calculated. Mean surviving fraction was calculated from at least three dishes.

3. RNA isolation and sequencing

The detailed protocols to seek underlying mechanism in radiosensitization of MA-17 are described in the previous study [9]. In brief, the cells were treated with media or an IC_{50}s concentration of DNMT inhibitors for 24 hours and irradiated with 6 Gy of 6 MV x-ray. Each samples were collected at 0, 2, 6, 24, 48 hours after radiation. After treatment, RNA isolation was performed. Total RNA concentration was calculated by Quant-IT RiboGreen (Invitrogen). To assess the integrity of the total RNA, samples are run on the TapeStation RNA screentape (Agilent). Only high-quality RNA preparations, with RIN greater than 7.0, were used for RNA library construction.

A library was prepared with 1ug of total RNA for each sample by Illumina TruSeq mRNA Sample Prep kit (Illumina, Inc., San Diego, CA, USA). The first step in the workflow involves purifying the poly-A containing mRNA molecules using poly-T-attached magnetic beads. Following purification, the

mRNA is fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments are copied into first strand cDNA using SuperScript II reverse transcriptase (Invitrogen) and random primers. This is followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. These cDNA fragments then go through an end repair process, the addition of a single 'A' base, and then ligation of the indexing adapters. The products are then purified and enriched with PCR to create the final cDNA library. The libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantificatoin kits for Illumina Sequecing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). Indexed libraries were then sequenced using the HiSeq2500 platform (Illumina,San Diego, USA by the Macrogen Incorporated.

4. Gene ontology (GO), Kegg pathway analysis (KPA)

We performed gene set analysis to do a functional study of the transcriptome data [14]. ToppGene was used to identify significantly enriched GO terms and pathways among the given list of genes that are differentially expressed in response to IR. Statistically overrepresented GO categories with Benjamini-Hochberg-adjusted p-value < 0.05 were considered significant. KEGG Mapper [15-17] was used to map

differentially expressed genes on KEGG pathways. 320 over-expressed genes with fold change >1.5 or 433 down-regulated genes with fold change <0.67 were used for this analysis.

5. Mechanism of radiosensitization

Flow cytometric assay was performed to identify the effect of drugs on cell cycles. Inhibitory effect on expression of DNMT1, DNMT3A/3B and expression of cleaved caspase-3, marker of apoptosis, were examined using Western blot for each proteins. Immunocytochemical analysis using the anti-γH2AX antibodies, a marker of double strand break, was performed to determine the effects of DNMT inhibitors on DNA repair.

6. Statistical analysis

Survival data was fitted using Kaleida graph version 3.51 (Synergy Software, Reading, PA, USA) into a linear quadratic (LQ) model. Differences in mean values between groups were compared using Student t-test. The clonogenic assay were analyzed using repeated measures one-way ANOVA. The Probability values of $p < 0.05$ were regarded as statistically significant. Statistical analysis was performed with the use of SPSS 23.0 statistical software.

Results

Effect of DNMT inhibitors on radiosensitivity of A549 cell line

The concentration of DNMT inhibitor, MA-17, PsA and Dacogen (5-aza-2' -deoxycytidine) had been titrated up to the 50% inhibitory concentrations (IC_{50}s) that reduce the viability of cancer cells after a 24 hours exposure to DNMT inhibitors. The IC_{50}s of DNMT inhibitors are as followed: MA-17 120 uM, PsA 5 uM, Dacogen 300nM. Comparison between survival curve of A549 cells treated with radiation and IC_{50}s of MA-17 and radiation alone was shown in Figure 1. The radiation dose required to generate a survival fraction (SF) of 0.5 and 0.2, the dose enhancement ratios (DER) were calculated. The DERs of MA-17 for the A549 cell line were 1.73 for SF 0.5 and 1.43 for SF 0.2, respectively.

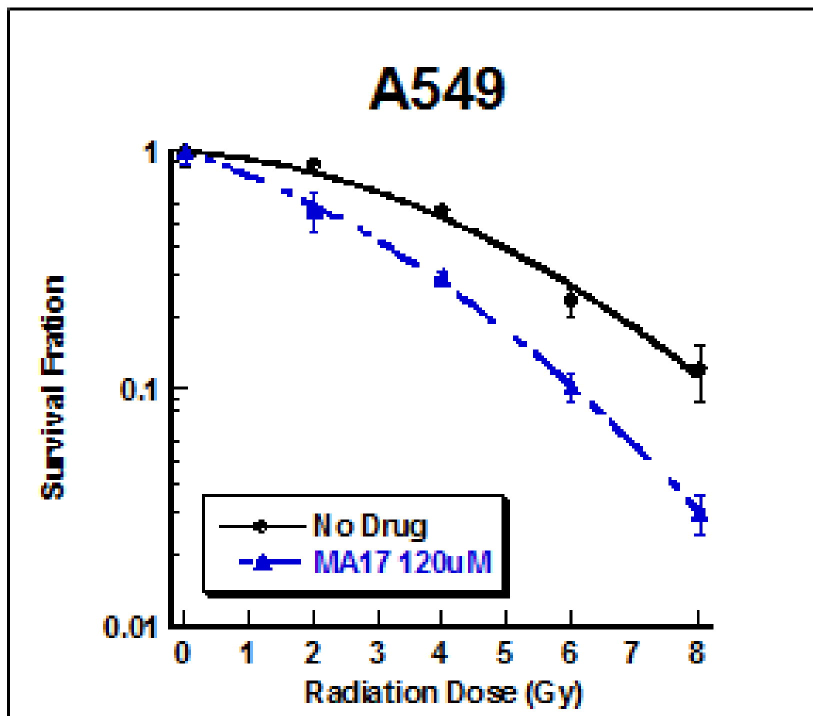


Figure 1. The effects of MA-17 on A549 cell radiosensitivity. Survival curves of A549 cells treated with MA-17 prior to radiation and radiation were compared with those of radiation alone. Points, mean for three independent experiments; bars, SE.

Transcriptome alteration modified by DNMT inhibitor in A549

To detect differences in genes and pathway affected by MA-17 pretreatment 24 hours before radiation, RNA sequencing was performed on A549 cells. Following 6 Gy irradiation, RNA was isolated at 6 hours prior to sequencing. To compare the radioenhancing effects of MA-17, a control sample was obtained after pretreatment of MA-17 24 hours before irradiation. The gene lists generated for irradiated cancer cells with or without MA-17 pretreatment at 6 hours following 6 Gy irradiation showed large differences in both the number and the type of genes that were transcriptionally activated. MA-17 appeared to impact the transcriptional response to a greater extent within the A549 cell line with severe significant differentially regulated genes by 6 hours (using a 1.5 fold cut-off).

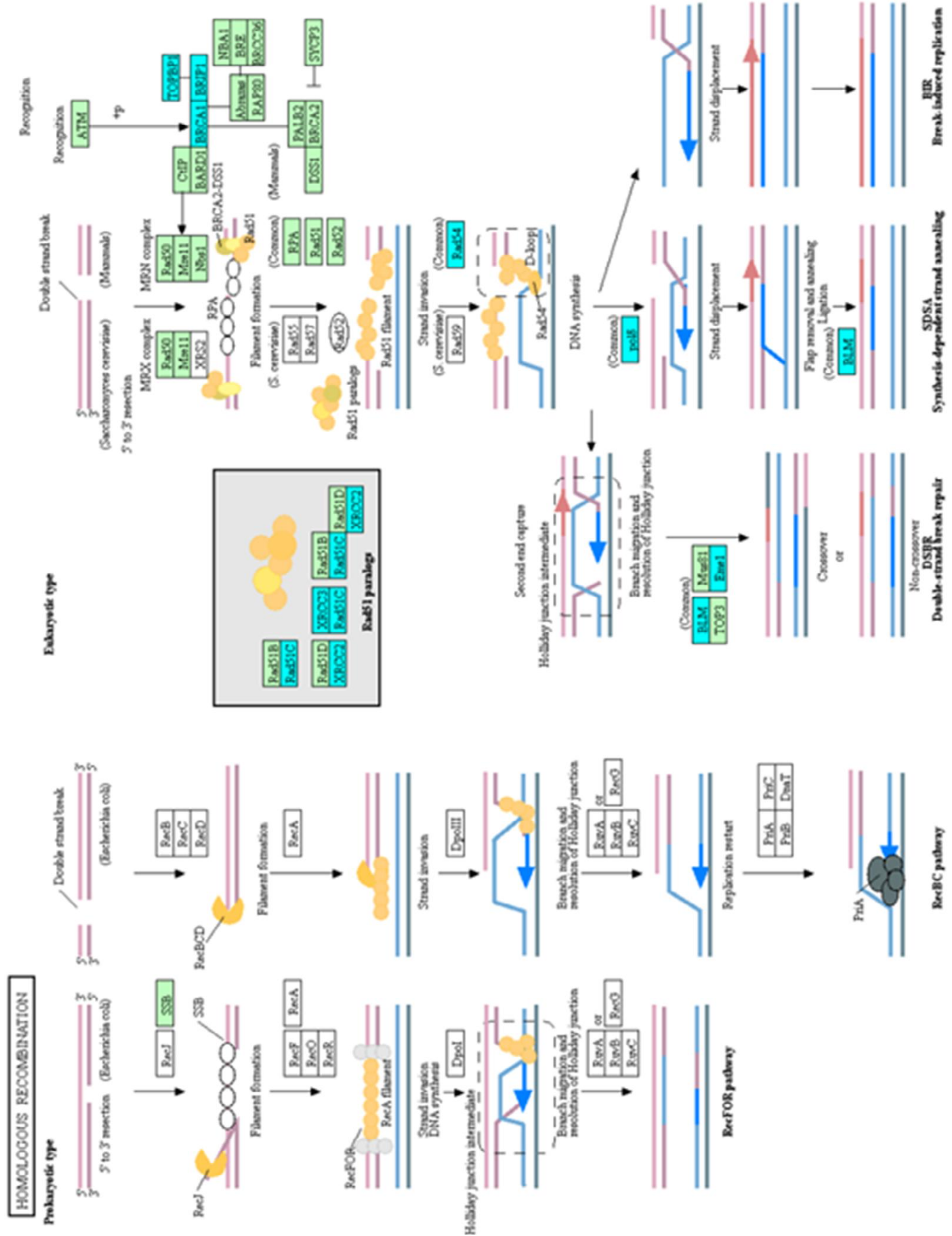
The Kegg pathway analysis was performed for functional analysis of our gene list acquired from the RNA sequencing analyses. Interactions between significant differentially regulated genes were screened and possible candidate hit-maps were selected. A total of 16 pathways were selected, which had several genes that were down-regulated from combined MA-17 and radiation compared radiation alone (Table 1). Two pathways relevant to DNA damage repair that were identified are shown in Figure 2: homologous recombination and the Fanconi anemia pathway.

Table 1. MA 17−associated pathways for radiosensitivity by Kegg pathway.

Kegg Pathway	**q−value FDR B&H**	**number of DEGs**	**number of genes**
Cell cycle	9.71E−18	28	124
DNA replication	1.78E−12	14	36
Homologous recombination	4.64E−08	11	41
Fanconi anemia pathway	1.12E−06	11	55
C5 isoprenoid biosynthesis, mevalonate pathway	2.29E−05	5	10
Cholesterol biosynthesis, squalene 2,3−epoxide => cholesterol	3.86E−05	5	11
Steroid biosynthesis	6.41E−05	6	20
Oocyte meiosis	1.10E−04	13	124
Mismatch repair	1.49E−04	6	23
Terpenoid backbone biosynthesis	1.38E−03	5	22
Pyrimidine metabolism	2.07E−03	10	105
Progesterone−mediated oocyte maturation	4.31E−03	9	96
Nucleotide excision repair	6.94E−03	6	47
Base excision repair	8.18E−03	5	33
p53 signaling pathway	1.01E−02	7	69
HTLV−I infection	1.07E−02	15	256

Abbreviations: FDR B&H, false discovery rate the Benjamini and Hochberg method; DEG, differentially expressed genes

A.



B.

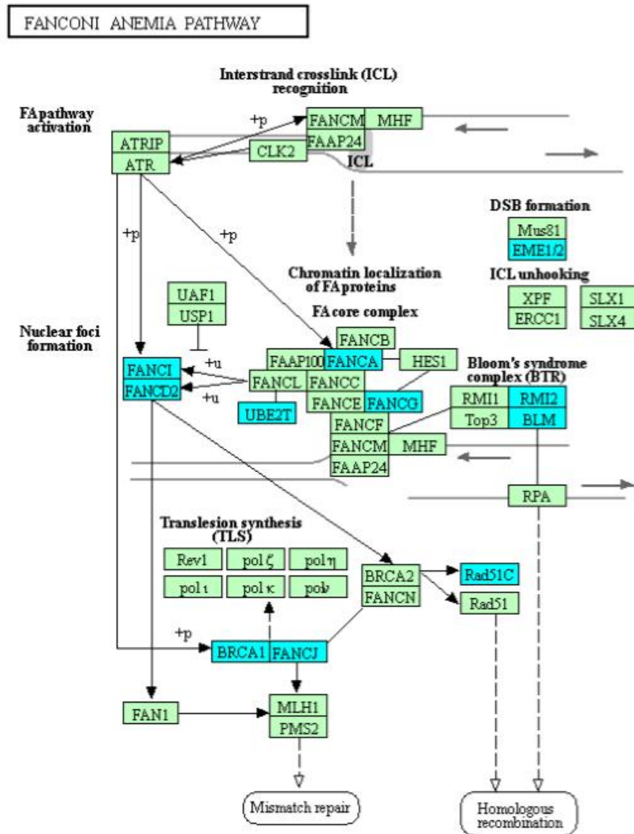


Figure 2. Pathways relevant to the effect of MA-17 on a radiosensitivity of A549 cells. Gene lists determined by RNA sequencing for A549 cell line after MA-17 and radiation combination treatment were analyzed using KEGG pathway analysis. Homologous recombination (A) and the Fanconi anemia (B) pathway were identified as being significantly downregulated by MA-17 and radiation. Significantly down-regulated genes are colored cyan , those present within our data set but not significant are shown in green. Significant genes were defined as reporting a fold change > 1.5 or <0.67.

Several genes have been demonstrated to have fold change >2 or <0.5 and they are highlighted in blue boxes. These regulated genes include FANCA, BRCA1, Rad51C. In additional gene set analysis to seek candidate genes those are related to the apoptotic pathway for radiosenstivity by MA-17, we found several genes with paramount DEG when treated with MA-17 (Table 2).

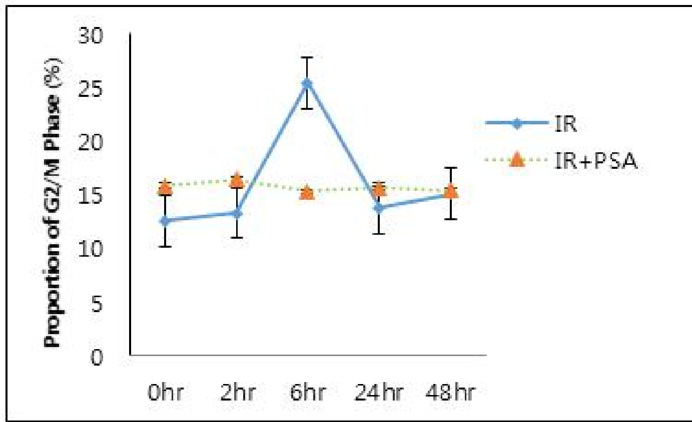
Mechanism of radiosensitization

Flow cytometry showed G2/M delay in A549 cells after radiation treatment alone. The effect of pretreatment of PsA was shown in Figure 3, radiation-induced G2/M arrest was abrogated at 6 hours. Whereas, delayed G2/M arrest at 24 hours was observed in cancer cells treated with MA-17 and radiation. Additionally, apoptotic rates was measured by the sub-G1 portion of flow cytometry, PsA and MA-17 pretreatment increased apoptotic rates when compared to cells irradiated alone. Increased cleaved caspase-3 protein level was observed in Western blotting in cells treated with a combination of DNMT inhibitors and radiation (Figure 4). The effect of combining Dacogen and radiation on the cell cycle and apoptosis was insignificant.

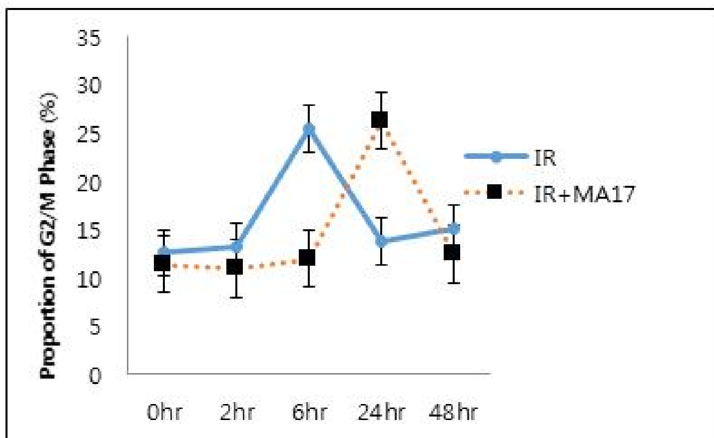
Table 2. List of genes in DEG analysis relevant to apoptosis of the Kegg pathway.

Gene	Log_2(fold change)
TNFRSF10D	0.868
JUN	0.836
BCL2A1	0.747
CTSL	0.700
BIRC3	0.664
MCL1	0.662

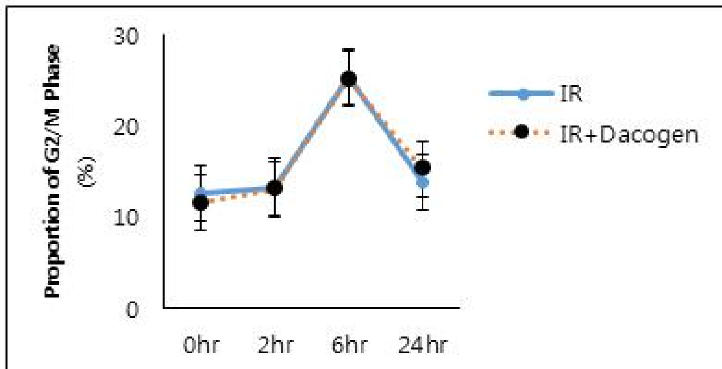
A.



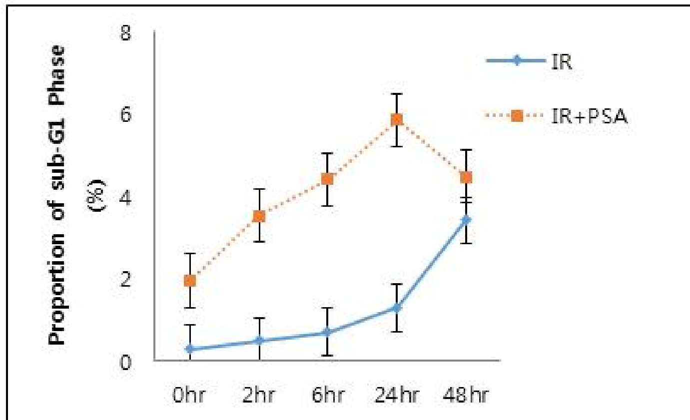
B.



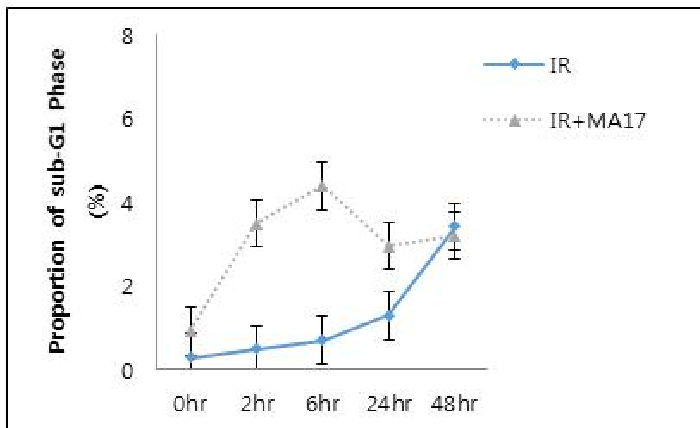
C.



D.



E.



F.

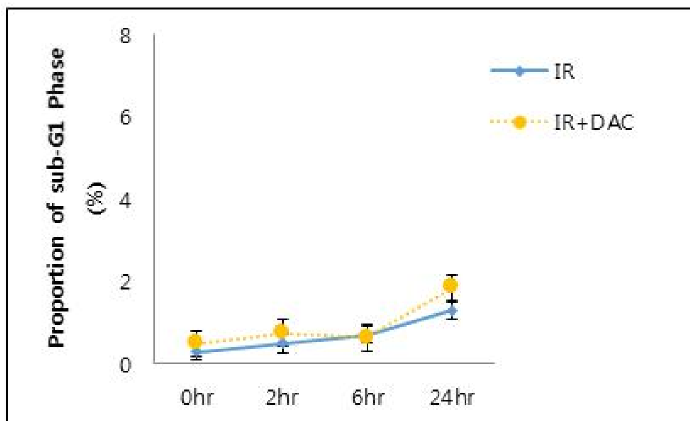
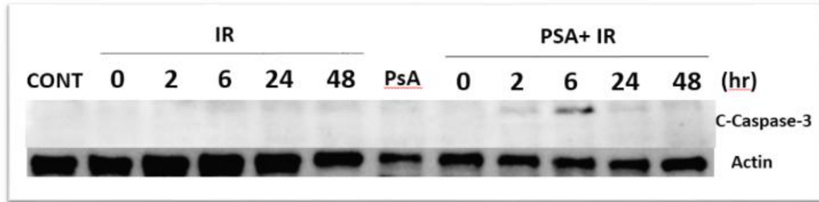


Figure 3. Influence of DNMT inhibitors on cell cycle phase distributions of A549 cells. Proportion of G2/M (A, B, C) and sub-G1 (D, E, F) of A549 cells treated with the respective DNMT inhibitors prior to radiation and radiation were compared with those of radiation alone. Points, mean for three independent experiments; bars, SE.

A.



B.

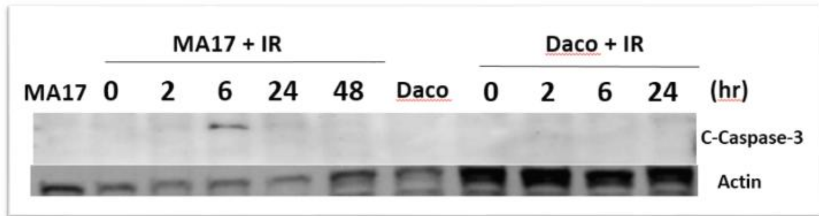


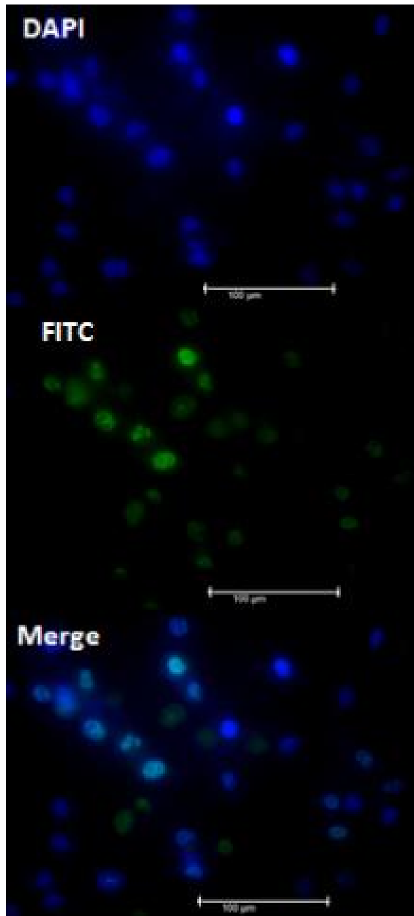
Figure 4. Western blot analysis of cleaved caspase-3. The cells were treated with a combination of DNMT inhibitors and 6 Gy of radiation. Increased cleaved caspase-3 protein level was observed in cells treated with a combination of 6 Gy of radiation and psammaplin A or MA-17 in the A549 cells.

Immunocytochemistry using the anti−γH2AX antibodies identified the effect of DNMT inhibibitors on DNA damage repair. γH2AX foci could be clearly visualized after radiation treatment (Figure 5) and the level of expression was shown to be reduced over time. In cells treated with combination of DNMT inhibitors and radiation, the level of γH2AX foci did not increase more at 1 - 6 hours, but it declined less at 24 - 48 hours, as compared to cells irradiated alone (Figure 6).

Validation of protein expression relevant to radiosensitization using DNMT inhibitor

From GO and Kegg pathway analysis, we found homologous recombination and the Fanconi anemia pathways are closely associated with radiosentizing effect of MA−17 on A549 cells. Based on the result, we chose the candidate genes for significant radiation enhancing factors when combined with MA−17 (RAD51C, BRCA1 and FANCA). To validate our sequencing data, expression levels following radiation treatment and MA−17 were evaluate using Western blotting. RAD51C, BRCA1, and FANCA expressions increased following radiation in the A549 cells with significant decrease in expression at 48 hours. In contrast, decreased expression of all proteins were observed after combined treatment of MA−17 and radiation. (Figure 7)

A.



B.

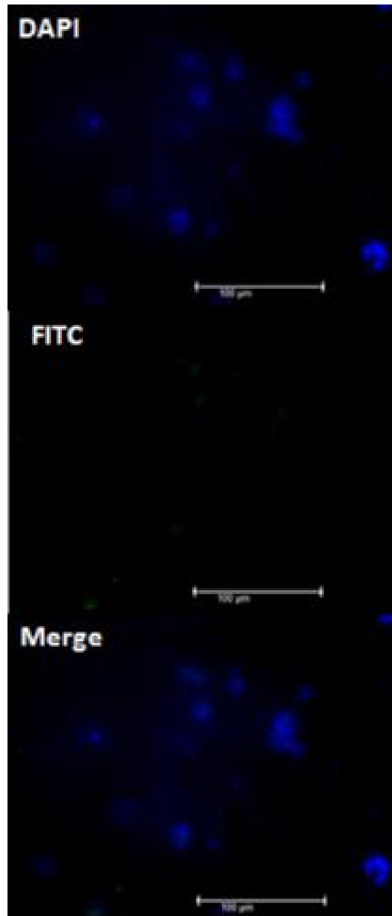
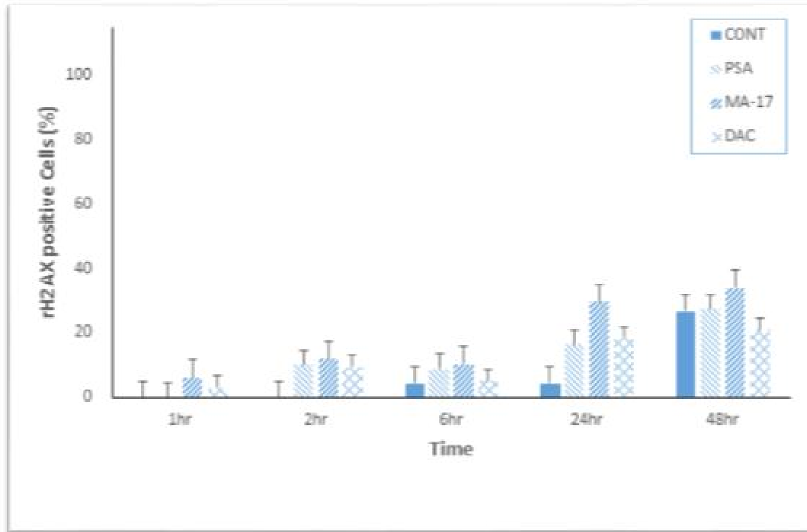


Figure 5. Radiation-induced γH2AX foci. Representative micrograghs were obrained from A 549 cells that had been treated with MA 17 and received 6 Gy radiation. γH2AX foci is clearly visualized at 48 hours after radiation treatment in co-treated cells (A). But the level of γH2AX expression declined drastically in cells treated with radiation alone at 48 hours after irradiation (B). DNA is visualized with DAPI, and merged images overlay γH2AX foci.

A.



B.

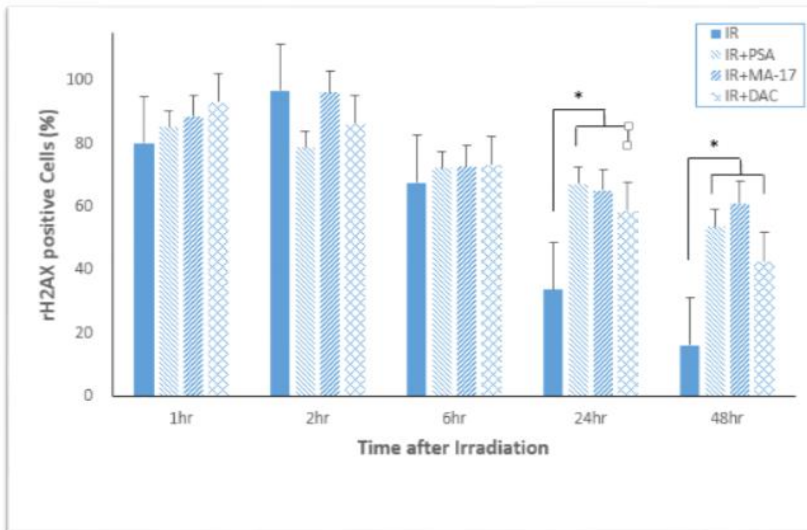


Figure 6. Influence of DNMT inhibitors on radiation-induced γH2AX foci. A549 cells were fixed after pretreatment of DNMT inhibitors and radiation. Cells with more than five foci per nucleus were classified as positive for radiation-induced γH2AX. Foci were evaluated in 50 nuclei. Bars, SE. *p < 0.01 as determined by a logistic regression compared with radiation alone (6 Gy) group.

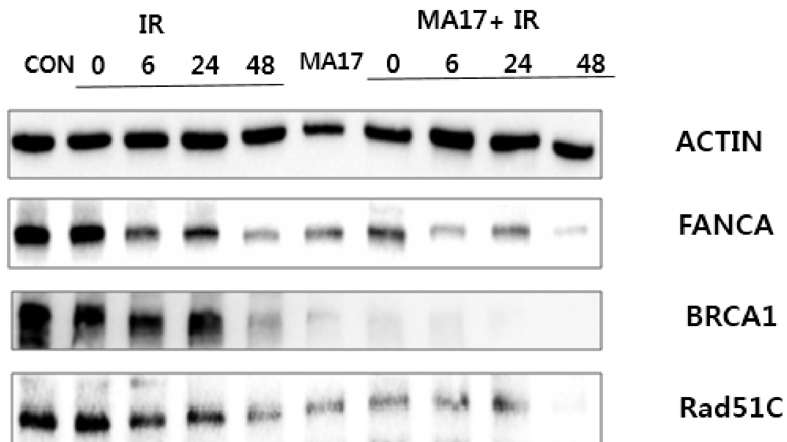


Figure 7. Western blot analysis of FANCA, BRCA1, Rad51C. Gene list was selected from candidate KEGG pathway analysis that supports in vitro results. Expression of three genes (FANCA, BRCA1, Rad51C) were validated using the Western blotting to compare between A549 cells treated with MA-17 prior to radiation and radiation versus those of radiation alone.

Effect of DNMT inhibitors on radiosensitivity of normal cell line

The IC_{50}s of DNMT inhibitors are determined for normal cell line (NHA) as followed: MA-17 300 uM, PsA 10 uM, Dacogen 300 nM. Comparison between survival curve of NHA cells treated with radiation combined with IC_{50}s of DNMT inhibitors and radiation alone was shown in Figure. Significant radiosensitivities of NHA cells after exposure to radiation and DNMT inhibitors were not demonstrated. The DERs of MA-17 for the A549 cell line were 1.02 for SF 0.5 and 1.11 for SF 0.2, respectively ($P > 0.05$)

Discussion

Radiation therapy is an established treatment modality for lung cancer, but radio−resistance is a major obstacle in cancer therapy. Dose−escalated radiation therapy can be tried to prove tumor control rates, but it is limited by the threshold dose of surrounding normal tissue that can be delivered without severe sequelae on non−target area. Any biologic modulator that enhances the radiation response without a dose escalation is advantageous in cancer treatment. Moreover, a radiosensitizer capable of exerting tumor−specific effect will improve the therapeutic index of radiotherapy. Enhancement of radiation response strategies have been successful in the treatment of a variety of cancers [18−21].

In our study, radio−sensitization by a novel synthetic DNMT inhibitor was observed in lung cancer cell line without affecting normal cell line. MA−17 significantly enhanced radiation−induced apoptosis and inhibition of the DNA damage repair. MA−17 has been demonstrated its value of clinical application as it has a relatively high bioavailability and a differential effect between cancer cells and normal cell line.

In general, DNMT inhibitor has been known to incorporate during the S−phase because of its nucleoside analog structure, and DNA synthesis also is inhibited as a result [22]. Thus

DNMT inhibitor can inhibit the repair of radiation induced DNA damage and they may reduce the number of tumor clonogens by a preferential cytotoxicity to proliferative cells [8]. In our previous research, we have demonstrated radiosensitizing effect of PsA, dacogen, and zebularine in lung cancer and glioblastoma cells by modulation of the impairment of the DNMA repair process. The expression of DNA damage marker as determined by γH2AX was increased after DNMT inhibitors and radiation co-treatment. This was confirmed in other in vitro [13] and in vivo [12] studies and reproduced in the present study. The cell cycle arrest abrogation is one of the possible mechanism for radiosensitization of DNMT though general inhibition of G2 checkpoint activation. An abrogation of radiation-induced G2/M arrest was noted in the PsA treated A549 cell line. But this effect was not shown in the MA-17 treated cells. Finally, there are evidences that DNMT inhibitors may trigger apoptosis. Qui et al. demonstrated that Dacogen induces increases in the apoptotic rate of gastric cancer cells and results in enhanced radio-sensitization [11]. De Schutter et al. also have reported the role of Dacogen on the head and neck cancer cells, inducing apoptosis when combined with radiation [23]. Similar results were noted in PsA and MA-17 pretreated A549 cells in this study.

Although some results allude to possible mechanisms of radiosensitization, no studies have shown definitive mechanisms.

To the best of our knowledge, this is the first presentation seeking possible mechanisms of radiosensitizing effect of DNMT inhibitor using transciptome data analysis. Using RNA sequencing, we identified radiation enhancing DNMT inhibitor induced transcriptome alteration in A549 cells. Comparing the RNA profiles altered by MA-17 and radiation combination, we found candidate genes with significant differential expression, up or down regulated by a novel radiosensitizer MA-17. Next we performed the GO and Kegg pathway analysis searching for significant and relevant molecular evidence. Several pathways were chosen for its statistical significance and two pathways relevant to DNA damage repair that were identified supporting our in vitro data.

The regulation of DNA repair and the Fanconi anemia related genes RAD51C, BRCA1 and FANCA correlated with radiation sensitivity in the A549 cells and this was validated in Western blotting. It has been well known that ionizing radiation induces various DNA damages [24,25], thus it is not surprising that down-regulation of DNA repair related genes enhances the radiation response. These genes are keys in regulating radiation response and the finding that epigenetic modulation by MA-17 enhances radiation response warrant further investigation. Also RAD51, BRCA1 and other FANC family gene FANCG have previously been reported to be associated with treatment responses in various cancers [26-28], but no research exists

investigating the association between radiosensitizer−induced down−regulation of these genes in radiation treatment of cancer cells. We cannot fully explain the mechanism of radiosensitization by these DNA repair pathway, however, our data implicate that DNA repair pathway might be possible therapeutic target for radiosensitization in NSCLC. Further study is needed to support our conclusion.

Taken together, the results of this study represent the first demonstration that a novel synthetic DNMT inhibitor MA−17 enhances radio−sensitization of lung cancer cells through inhibition of repair of DNA DSB and enhanced apoptosis. Furthermore, the Fanconi anemia pathway could be involved in the underlying mechanism of the combination treatment. These findings not only confirm that targeting DNA damage repair remains an important therapeutic strategy for the radiosensitization of lung cancer, but also suggest a new application of an epigenetic drug as an enhancing agent for radiotherapy.

References

1. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683−692.
2. Baylin SB, Schuebel KE. Genomic biology: The epigenomic era opens. Nature 2007;448:548−549.
3. Rodriguez−Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nat Med 2011;17:330−339.
4. Turner BM. Defining an epigenetic code. Nat Cell Biol 2007;9:2−6.
5. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood 2015;127:42−52.
6. Nervi C, De Marinis E, Codacci−Pisanelli G. Epigenetic treatment of solid tumours: A review of clinical trials. Clin Epigenetics 2015;7:127.
7. Merrifield M, Kovalchuk O. Epigenetics in radiation biology: A new research frontier. Front Genet 2013;4:40.
8. Smits KM, Melotte V, Niessen HE, Dubois L, Oberije C, Troost EG, Starmans MH, Boutros PC, Vooijs M, van Engeland M, Lambin P. Epigenetics in radiotherapy: Where are we heading? Radiother Oncol 2014;111:168−177.
9. Kim HJ, Kim JH, Chie EK, Young PD, Kim IA, Kim IH. Dnmt (DNA methyltransferase) inhibitors radiosensitize human cancer cells by suppressing DNA repair activity. Radiat Oncol 2012;7:39.
10. Kim HJ, Kim TH, Seo WS, Yoo SD, Kim IH, Joo SH, Shin S, Park ES, Ma ES, Shin BS. Pharmacokinetics and tissue distribution of

psammaplin a, a novel anticancer agent, in mice. Arch Pharm Res 2012;35:1849–1854.

11. Qiu H, Yashiro M, Shinto O, Matsuzaki T, Hirakawa K. DNA methyltransferase inhibitor 5–aza–cdr enhances the radiosensitivity of gastric cancer cells. Cancer Sci 2009;100:181–188.
12. Dote H, Cerna D, Burgan WE, Carter DJ, Cerra MA, Hollingshead MG, Camphausen K, Tofilon PJ. Enhancement of in vitro and in vivo tumor cell radiosensitivity by the DNA methylation inhibitor zebularine. Clin Cancer Res 2005;11:4571–4579.
13. Wang L, Zhang Y, Li R, Chen Y, Pan X, Li G, Dai F, Yang J. 5–aza–2'–deoxycytidine enhances the radiosensitivity of breast cancer cells. Cancer Biother Radiopharm 2013;28:34–44.
14. Chen J, Bardes EE, Aronow BJ, Jegga AG. Toppgene suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res 2009;37:W305–311.
15. Kanehisa M, Goto S. Kegg: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28:27–30.
16. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. Kegg as a reference resource for gene and protein annotation. Nucleic Acids Res 2016;44:D457–462.
17. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. Kegg: New perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45:D353–D361.
18. Jassem J. Combined chemotherapy and radiation in locally advanced non–small–cell lung cancer. Lancet Oncol 2001;2:335–342.
19. Vongtama V, Douglass HO, Moore RH, Holyoke ED, Webster JH. End results of radiation therapy, alone and combination with 5–fluorouracil in colorectal cancers. Cancer 1975;36:2020–2025.

20. Lo TC, Wiley AL, Jr., Ansfield FJ, Brandenburg JH, Davis HL, Jr., Gollin FF, Johnson RO, Ramirez G, Vermund H. Combined radiation therapy and 5-fluorouracil for advanced squamous cell carcinoma of the oral cavity and oropharynx: A randomized study. AJR Am J Roentgenol 1976;126:229-235.
21. Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs CL, 3rd, Walker JL, Gersell D. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage ib cervical carcinoma. N Engl J Med 1999;340:1154-1161.
22. Song SH, Han SW, Bang YJ. Epigenetic-based therapies in cancer: Progress to date. Drugs 2011;71:2391-2403.
23. De Schutter H, Kimpe M, Isebaert S, Nuyts S. A systematic assessment of radiation dose enhancement by 5-aza-2'-deoxycytidine and histone deacetylase inhibitors in head-and-neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2009;73:904-912.
24. Prise KM, Schettino G, Folkard M, Held KD. New insights on cell death from radiation exposure. Lancet Oncol 2005;6:520-528.
25. Gatti RA. The inherited basis of human radiosensitivity. Acta Oncol 2001;40:702-711.
26. Nogueira A, Catarino R, Faustino I, Nogueira-Silva C, Figueiredo T, Lombo L, Hilario-Silva I, Pereira D, Medeiros R. Role of the rad51 g172t polymorphism in the clinical outcome of cervical cancer patients under concomitant chemoradiotherapy. Gene 2012;504:279-283.
27. Casado JA, Rio P, Marco E, Garcia-Hernandez V, Domingo A, Perez L, Tercero JC, Vaquero JJ, Albella B, Gago F, Bueren JA.

Relevance of the fanconi anemia pathway in the response of human cells to trabectedin. Mol Cancer Ther 2008;7:1309–1318.
28. Papadaki C, Sfakianaki M, Ioannidis G, Lagoudaki E, Trypaki M, Tryfonidis K, Mavroudis D, Stathopoulos E, Georgoulias V, Souglakos J. Ercc1 and brac1 mrna expression levels in the primary tumor could predict the effectiveness of the second–line cisplatin–based chemotherapy in pretreated patients with metastatic non–small cell lung cancer. J Thorac Oncol 2012;7:663–671.

초 록

서론: 후성유전학적 변화는 유전자 조절의 중요한 메커니즘이다. 이중 DNA 메틸기 전달효소 (DNMT)의 조절은 방사선 조사 후 세포 반응과 관련된 주요 기작이며 이를 타겟으로 하는 DNMT 저해제는 방사선 감작제 후보물질로 알려져 왔다. 본 연구에서는 새로운 DNMT 저해제인 MA-17의 방사선 감작 효과를 알아보고자 한다.

방법: DNMT 저해제 MA-17을 방사선 조사 전에 처치했을 때의 효과를 평가하기 위해, 폐암 세포주인 A549 세포를 배양하여 대상 세포생존을 결정하기 위한 세포집락 검사 (clonogenic assay)를 시행하였다. MA-17의 방사선감작효과와 관련된 메커니즘과 MA-17 전처치 여부에 따른 유전자 발현의 차이를 유전자 온톨로지 및 신호전달 체계에서 확인하였다. 세포사의 방식이 세포고사임을 확인하기 위한 caspase-3 assay을, 이중나선 절단 회복 지연에 관여하는지 확인하기 위한 γ H2AX의 면역화학염색 등을 시행하였다. 유세포분석을 통하여 세포주기 및 세포고사 관련된 세포 주기 분포의 변화를 분석하였다.

결과: 방사선 조사 전 120uM의 MA-17 전처치는 A549 세포에 대한 방사선 감작 효과를 나타내었으며, 생존 분획 0.5에 해당하는 감작 증강 비율은 1.73이었다. 차세대 염기서열 분석을 통하여 MA-17 병용 처치 시 DNA 상동 재조합 및 Fanconi anemia 전달체계 발현을 억제가 관여하고, 세포고사를 증가시킴을 확인하였다. 이에 대한 검증 실험에서는 MA-17 병용처치 후 caspase-3 발현이 증가하였는데, 이는 유세포 검사에서 Sub-G1 주기 증가로도 확인 되었다. 또한 방사선 단독 처치에 비해 MA-17 전처치한 경우 γH2AX 발현 정도가 48시간 경과 후에도 높게 유지되었으며, 이는 이중나선 절단 회복의 지연이 방사선 감작 효

과의 주요 메커니즘임을 시사한다. 이러한 A549의 방사선치료와 MA-17의 세포살상 효과 증강은 정상 교세포에서는 관찰되지 않았다. **결론:** DNMT 저해제인 MA-17이 폐암 세포주인 A549에서 방사선조사와 병용 시에 세포치사 효과를 향상시켰다. 이 방사선 감작 효과는 DNA 이중나선 절단의 회복을 억제하고 세포고사를 증가시키는 효과를 통해 이루어진다.

주요어: 폐암, 방사선 감수성, DNMT 저해제, 후성 유전학
학 번: 2010-30495