Proton Irradiation Sensitizes Radioresistant Non-small Cell Lung Cancer Cells by Modulating Epidermal Growth Factor Receptor-mediated DNA Repair

HYUNG JU PARK[1*], JEONG SU OH[2*], JONG WOOK CHANG[3], SANG-GU HWANG[4] and JAE-SUNG KIM[4*]

[1]*IT Convergence Technology Research Laboratory, Electronics and Telecommunications Research Institute, Daejeon, Republic of Korea;*
[2]*Department of Genetic Engineering, Sungkyunkwan University, Suwon, Republic of Korea;*
[3]*Stem Cell Regenerative Medicine Center, Research Institute for Future Medicine, Samsung Medical Center, Seoul, Republic of Korea;*
[4]*Division of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea*

Abstract. *Although proton radiotherapy is effective in treating various types of cancer, little is known on the biological responses triggered by proton irradiation. In the present study, we investigated protein profiles following proton irradiation of non-small cell lung cancer (NSCLC) cells and defined the role of proton-induced epidermal growth factor receptor (EGFR) expression in NSCLC cells. We found that proton irradiation more effectively sensitized NSCLC cells than gamma irradiation did. The expression profiles of radiosensitive and radioresistant NSCLC cells following proton and gamma irradiation were examined using antibody arrays. With regard to proteins, expression of EGFR was the most highly induced by proton irradiation. In addition, we found that EGFR inhibition with gefinitib significantly increased the radiosensitivity of NSCLC cells, and that increased radiosensitivity due to gefinitib was mediated by the suppression of DNA repair in radioresistant NSCLC cells. Thus, our data provide the first evidence that proton irradiation sensitizes radioresistant NSCLC cancer cells by modulating EGFR-mediated DNA repair.*

Lung cancer is the leading cause of cancer-related death worldwide. About 85-90% of lung cancer diagnoses are non-small cell lung cancer (NSCLC) (1). Radiotherapy is an essential treatment modality for early-stage and locally advanced NSCLC. However, current treatment delivery is often limited by radiation doses that can penetrate non-tumor tissue and treatment-related toxicities such as pneumonitis and fibrosis (2). Proton radiotherapy, controlled through its characteristic Bragg peak, can reduce the toxicity of radiotherapy and consequently improve patient outcomes (3, 4). Although proton radiotherapy has been effective in treating several types of cancers, including lung cancer, the biological effects of proton radiation are still not well understood.

Epidermal growth factor receptor (EGFR) promotes the activation of survival signaling pathways such as phosphoinositide 3-kinase/AKT (PI3K/AKT) and RAS-mitogen-activated protein kinases (5). Increased EGFR activation and overexpression in various types of cancers is closely associated with tumorigenesis and cancer progression (6). Thus, EGFR is an important target for anticancer treatment, and particularly of lung cancer (6). *In vitro* and *in vivo* data report synergism between EGFR inhibitors, *e.g.* gefitinib, and radiation success (7-9), indicating that EGFR inhibition enhances the efficacy of radiotherapy (10). Radiation can directly activate EGFR signaling to promote cell survival and activate DNA double-strand break (DSB) repair (11, 12). Thus, these mechanisms suggest that EGFR overexpression triggered by irradiation is linked to tumor resistance to radiotherapy (13, 14). Although the role of EGFR in radiation therapy is well established, the role of EGFR in response to proton radiotherapy is unknown.

The biological effects of proton irradiation were previously assumed to be similar to those induced by photon radiation, such as gamma radiation (4). However, accumulating evidence indicates different biological responses to proton and photon (*e.g.* gamma) irradiation in cells (4, 15-17). In this

*These Authors contributed equally to this study.

Correspondence to: Jae-Sung Kim, Division of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, 215-4 Gongneung-Dong, Nowon-Ku, Seoul 139-706, South Korea. Tel: +82 29701669, Fax: +82 29702417, e-mail: jaesung@kirams.re.kr

Key Words: proton radiation, gamma radiation, lung cancer, EGFR, DNA repair.

study, the biological effects and protein profiles following proton irradiation were compared to the responses to gamma irradiation in radiosensitive and radioresistant NSCLC cells. Our data provide evidence that proton radiotherapy effectively sensitizes radioresistant NSCLC cells by modulating EGFR-mediated DNA-repair capacity.

Materials and Methods

Cell lines and treatment. Human NSCLC H460 and H1299 cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) and penicillin/ streptomycin at 37˚C in a humidified 5% CO_2 atmosphere. Gefinitib (1 μM; AstraZeneca, Wilmington, DE, USA) was used to inhibit EGFR activity.

Irradiation. For gamma irradiation, the cells were irradiated using a ^{137}Cs ray source (Atomic Energy of Canada Ltd., Mississauga, Canada) at a dose rate of 3.81 Gy/min. For proton irradiation, the cells were irradiated with a 35-MeV proton beam using the MC-50 cyclotron (Scanditronix, Uppsala, Sweden) according to a previous report (18). Cells seeded in 12.5-cm^2 flasks or 60-mm tissue culture dishes were placed on a beam stage and then irradiated at the center of Bragg peaks adjusted to a width of 6 cm. A mono-energetic proton beam could not be applied to cancer cells because the Bragg peak was too narrow to give a uniform dose to a tumor of any significant depth. Thus, a spread-out Bragg peak dose distribution was created by traversing a rotating range modulator designed to deliver a uniform dose distribution to a specified depth in cells plated and in media. The average dose rate was 2.31 Gy/s. Radiochromic film (GAF-MD55) (International Specialty Products, Wayne, NJ, USA) was used as a tool for *in situ* measurement of the dose at each beam irradiation.

Cell-death analysis. Cell death was analyzed as described previously (19). Briefly, cells were trypsinized and washed, and then incubated with propidium iodide (5 μg/ml) for 10 min. The cells were analyzed with a fluorescence-activated cell sorting (FACS) flow cytometer (Becton Dickson, Franklin Lakes, NJ, USA).

Clonogenic assay. Cell survival after irradiation was determined by a clonogenic assay as described previously (19). Briefly, various densities of cells treated with different doses of radiation were plated in triplicate in 60-mm tissue culture dishes. After 10-14 days the colonies were fixed with methanol and stained with trypan blue solution. Only colonies containing more than 50 cells were counted as surviving colonies.

Antibody array. Freeze-dried protein samples of untreated H1299 and H460 cells (Ctrl) and cells treated with 6-Gy proton or gamma irradiation were prepared for antibody array and analyzed by E-biogen (www.e-biogen.com, Seoul, South Korea). Briefly, signal explorer antibody microarray slides (Fullmoon Biosystems, Sunnyvale, CA, USA), coated with 1,358 antibodies, were treated with blocking solution and incubated on a shaker for 45 min. After blocking, the slides were rinsed with Milli-Q grade water. The labeled samples were mixed in 6 ml of coupling solution. The blocked array slides were then incubated with the coupling mixture on a shaker at 60 rpm for 2 h in a coupling dish. After coupling, the slides were washed six times with 30 ml of washing solution. Subsequently, 30 μl of 0.5 mg/ml Cy3-streptavidin (GE Healthcare, Chalfont St. Giles, UK) was mixed with 30 ml of detection buffer. The slides were dried completely before scanning within 24-48 h using a GenePix 4000B scanner (Axon Instrument Inc., CA, USA). The scanned images were quantified using GenePix Software (Axon Instrument), and the numeric data were analyzed using Genowiz 4.0™ (Ocimum Biosolutions Ltd., Hyderabad, India). After analysis, the protein data were annotated using UniProt DB (available at www.uniprot.org).

Western blot analysis. Western blotting was performed as described previously (19, 20). Briefly, proteins from untreated H1299 and H460 cells (Ctrl) and cells treated with 6-Gy proton or gamma irradiation were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and then detected using specific antibodies. The following antibodies were used: rabbit polyclonal anti-phosphatase and tensin homolog (PTEN) and rabbit monoclonal anti-phospho-AKT from Epitomics (Burlingame, CA, USA); rabbit polyclonal anti-cleaved caspase-3 and rabbit polyclonal anti-AKT from Cell Signaling Technology (Beverly, MA, USA); mouse monoclonal anti-p53, mouse monoclonal anti-p21, rabbit polyclonal anti-EGFR, and anti-human epidermal growth factor receptor 2 (HER2) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA); and mouse monoclonal anti-β-actin from Sigma (St. Louis, MO, USA). Blots were developed using horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence detection system (Amersham Life Science, Piscataway, NJ, USA).

Immunofluorescent staining for γ-H2AX. Immunofluorescent staining was performed as described previously (21). Cells grown on coverslips in 35-mm dishes were treated 3 Gy proton- or gamma irradiation and then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). The cells were permeabilized and blocked with 0.2% Triton X-100 and 5% FBS in PBS. These fixed cells were then incubated consecutively with primary antibodies against γ-H2AX (1:500; Millipore) and secondary antibody, anti-mouse Alexa-488 (Molecular Probes, Eugene, OR, USA). Slides were mounted in medium containing 4',6-diamidino-2-phenylindole (DAPI), and images were then obtained using a confocal laser-scanning microscope (LSM 710; Carl Zeiss, Inc., Oberkochen, Germany). γ-H2AX foci were quantified using CellProfiler software (available at www.cellprofiler.org).

Statistical analysis. A two-tailed Student's *t*-test was used to determine statistical differences between groups. A value of $p<0.05$ was considered statistically significant.

Results

Proton irradiation more effectively sensitized NSCLC cells than gamma irradiation. Since recent reports suggested that proton irradiation induces increased apoptosis compared to gamma irradiation in various cells and mice models (17, 21-23), we first examined whether proton irradiation is more potent in sensitizing NSCLC cells compared to gamma irradiation. Furthermore, in order to understand the differential effects of proton irradiation between

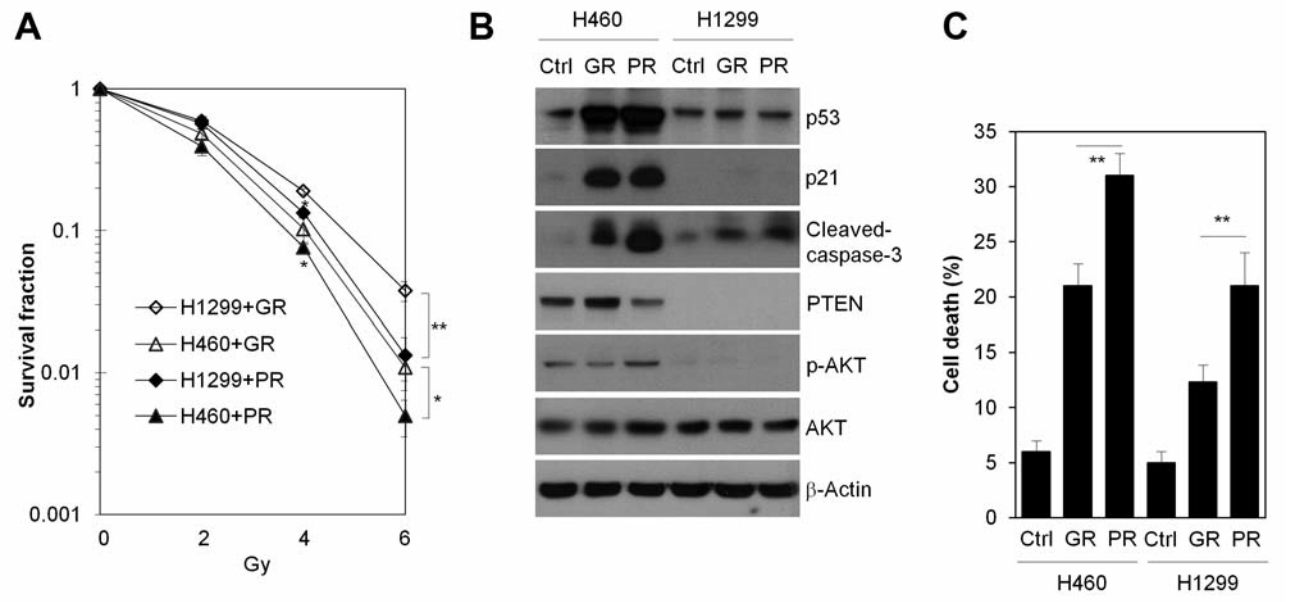

Figure 1. *Proton irradiation more effectively radiosensitized non-small cell lung cancer cells than did gamma irradiation. A: H460 and H1299 cells were treated with different doses of proton (PR) or gamma radiation (GR), as indicated. The clonogenic survival fraction was determined by clonogenic assay. B: H460 and H1299 cells were analyzed by immunoblotting with the indicated antibodies. β-Actin was used as a loading control. C: H460 and H1299 cells were either left untreated (Ctrl) or treated with 6 Gy of PR or GR for 48 h. Cell viability was determined with a FACScan flow cytometer and data are presented as a percentage of propidium iodide-positive cells and are the mean±standard deviation of three independent experiments. *p<0.05 And **p<0.01 compared to gamma-irradiated control cells.*

radiosensitive and radioresistant cells, we used two NSCLC cells lines, intrinsically radioresistant H1299 cells and intrinsically radiosensitive H460 cells (Figure 1A) (24). Clonogenic analysis revealed that proton irradiation was more effective at sensitizing NSCLC cells compared to gamma irradiation (Figure 1A). Interestingly, we also found that the radiosensitizing effect of proton irradiation in H1299 cells was greater than the effects in H460 cells (Figure 1A). In addition, several signaling molecules that regulate apoptosis and survival pathways such as p53 and PI3K/AKT were differentially regulated by 6 Gy of gamma *vs*. proton radiation (Figure 1B). Proton irradiation induced more cell death than gamma irradiation, as evident by the increased cleaved-caspase-3 observed in the FACS analysis (Figure 1B and C). Therefore, these results indicated that proton irradiation more potently triggered cell death than did gamma irradiation in NSCLC cells.

Expression profiles in radiosensitive and radioresistant lung cancer cells differ after proton and gamma irradiation. Next, in order to find the key factors regulating the proton-mediated radiation response, the differential signal profiles following proton and gamma irradiation were investigated in H1299 and H460 cells using a signal explorer antibody array chip coated with 1,358 antibodies. Antibody array analysis was performed using untreated H1299 and H460 cells (Ctrl) and cells treated with 6 Gy proton or gamma irradiation. The significantly regulated signaling proteins (up- or down-regulated by more than 1.5-fold) were selected. Relative to the control, 79 out of 1,358 proteins were down-regulated and 89 out of 90 proteins were up-regulated in gamma- and proton-irradiated H460 cells, respectively (data not shown). A total of 147 and 65 proteins were down-regulated, and 140 and 42 proteins were up-regulated in gamma- and proton-irradiated H1299 cells, respectively (data not shown). Similar to observations by other groups (4, 15, 23, 25), we found that signaling proteins associated with inflammation, cellular defense response, apoptosis, and the cell cycle were commonly affected by proton and gamma irradiation (data not shown). Nevertheless, many signaling proteins were differentially regulated between gamma- and proton-irradiated cells (Figure 2). In addition, more differentially regulated proteins were found in H460 cells than in H1299 cells (Figure 2), suggesting that the response to both proton and gamma irradiation is greater in radiosensitive H460 cells. Therefore, these observations suggest that proton irradiation triggers many different signal responses compared to gamma irradiation.

Increased EGFR expression in response to proton irradiation regulated radioresistance of NSCLC cells. Out of the differential signaling profiles, several signaling protein families, including EGFR, caspase, cyclins, and claudins,

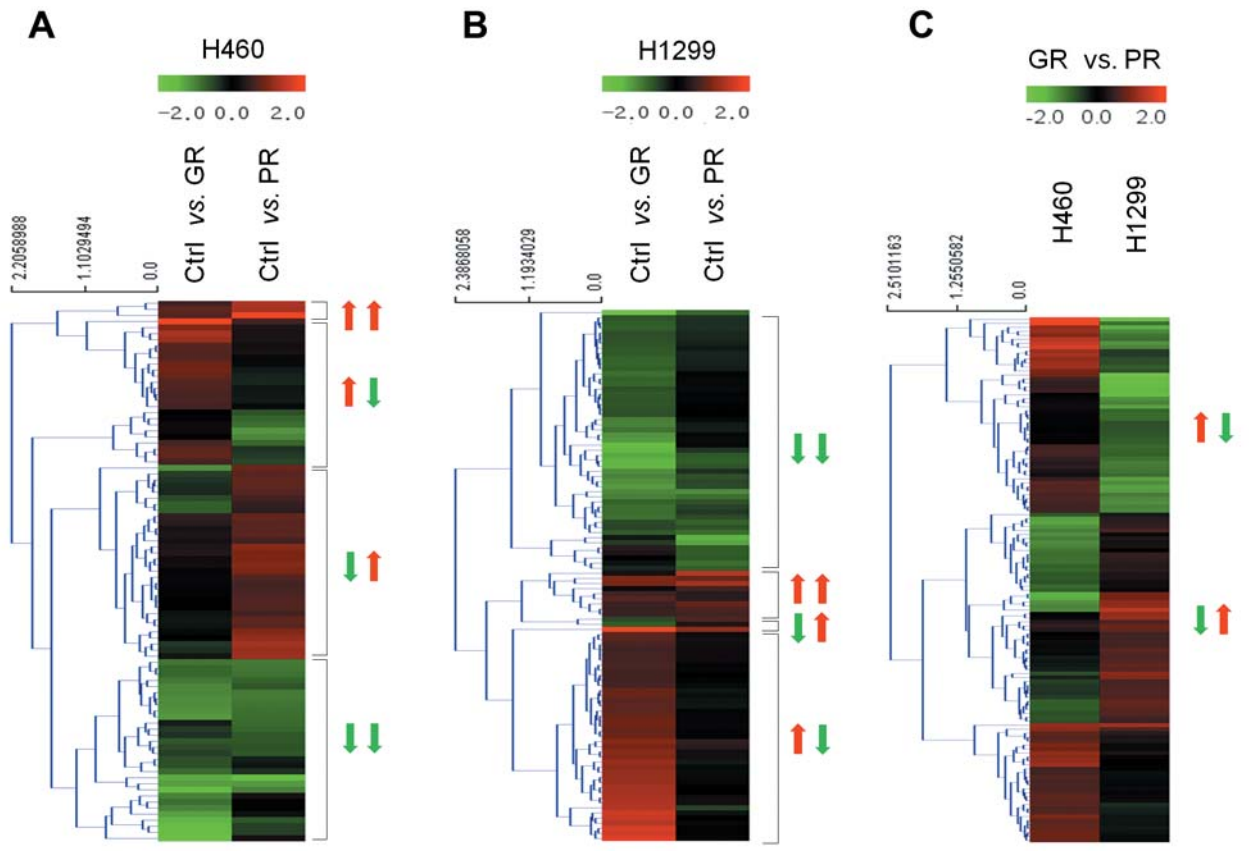

Figure 2. *Heatmap analysis of the antibody array of proton- and gamma-irradiated H460 and H1299 cells. H460 (A) and H1299 (B) cells were either untreated (Ctrl) or treated with 6 Gy of proton (PR) or gamma (GR) radiation for 24 h. The protein samples of treated cells were subjected to antibody arrays. The differentially regulated proteins (>1.5-fold) were quantified and are presented in a heat map which indicates antibody reactivity intensity as red pixels (values >1.5-fold) and as green pixels (values <1.5-fold). C: The differentially regulated proteins following proton vs. gamma irradiation in H460 and H1299 cells are presented in a heat map. Green and red arrows indicate down-regulation and up-regulation of protein expression, respectively.*

were quantified (Figure 3). Since EGFRs that are highly expressed in NSCLC have been implicated in tumor development and are regarded as an important target for NSCLC therapy (6), we validated the increased EGFR expression in response to proton irradiation. Consistent with the protein-array data, EGFR was significantly induced by proton irradiation compared to gamma irradiation in both H460 and H1299 cells, while HER2 was not (Figure 4A). To investigate the role of proton-induced EGFR expression, gefitinib, an EGFR tyrosine kinase inhibitor, was used as a co-treatment with gamma and proton irradiation in both H460 and H1299 cells. An analysis of survival, the rate of colony formation in response to 3 Gy of radiation, indicated that gefitinib significantly sensitized both H460 and H1299 cells to proton irradiation relative to gamma irradiation (Figure 4B and C). Interestingly, the sensitizing effect of proton irradiation combined with gefitinib was more potent in H1299 than in H460 cells (Figure 4C). Therefore, these results suggest that proton-mediated EGFR expression regulates the radioresistance of NSCLC cells, and that combined treatment of proton radiation and an EGFR inhibitor could enhance the efficacy of proton therapy in NSCLC.

EGFR inhibition showed greater suppression of DNA-repair capacity for radiation-induced DSBs in proton-irradiated NSCLC cells. Next, the DNA repair capacity in radioresistant H1299 cells was examined by quantifying γ-H2AX foci, that indicate radiation-induced DSBs (26), since it is known that EGFR regulates radioresistance by modulating DNA repair following ionizing irradiation (8). γ-H2AX foci were clearly detected in 3 Gy proton- or gamma-irradiated cells. The average numbers of γ-H2AX foci per cell were assessed at different time points (Figure 5). The average number of γ-H2AX foci in the gefinitib plus irradiation-treated cells was significantly higher at the 0.5 h time point than that of control cells only gamma- or proton-irradiated (Figure 5),

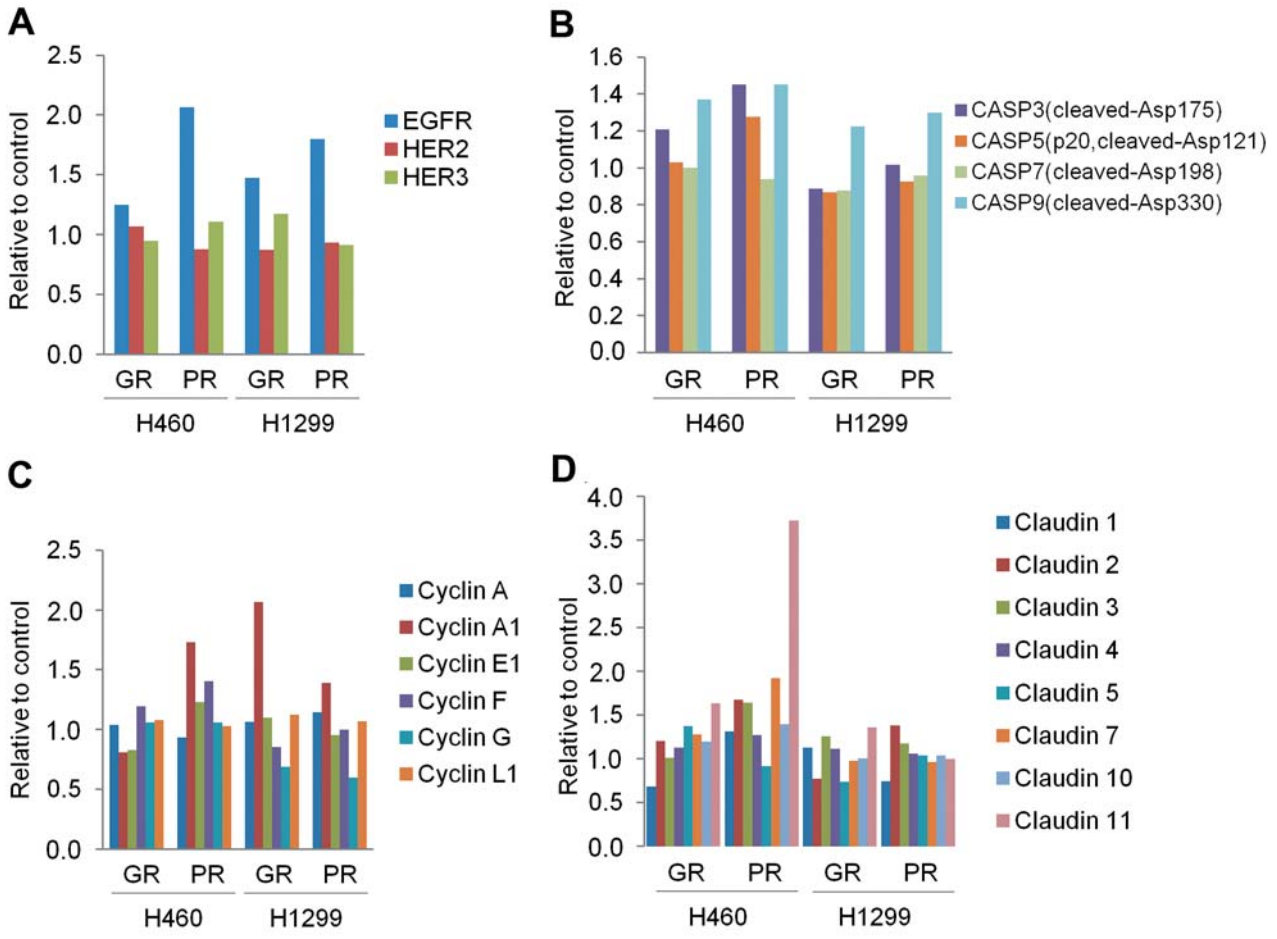

Figure 3. *Quantification of expression of epidermal growth factor receptor (EGFR) family (A), caspase family (B), cyclin isoform (C) and claudin family (D) in proton- (PR) and gamma-irradiated (GR) H460 and H1299 cells. Expressions were quantified based on the antibody array analysis as described in the Material and Method section. HER: Human epidermal growth factor receptor.*

indicating that EGFR regulates DNA-repair activity in response to radiation. Importantly, we also found that proton irradiation suppressed DNA-repair capacity to a greater extent than did gamma irradiation (Figure 5B), implying that proton irradiation more profoundly inhibited DNA-repair capacity. Taken together, these results suggest that proton-mediated EGFR expression modulates DNA-repair capacity, leading to radioresistance of NSCLC cells.

Discussion

Although proton radiotherapy is increasingly used in anticancer treatments due to its superior targeting of the tumor site, the biological responses to proton irradiation are not well understood. The present study investigated the signaling-protein profiles for proton irradiation in radioresistant and radiosensitive NSCLC cells, and provides evidence that proton radiation-mediated EGFR expression modulates DNA-repair capacity, contributing to the radiation resistance of NSCLC cells. Furthermore, our data show that proton irradiation combined with gefitinib enhances the sensitivity of radioresistant NSCLC cells, suggesting that proton radiotherapy with EGFR inhibitor treatment increases the efficacy of proton radiotherapy in NSCLC.

Superior sensitizing effects of proton irradiation have been reported by other groups (3, 4). Proton irradiation is a more potent inducer of apoptotic cell death compared to gamma irradiation in leukemia, prostate cancer, and melanoma cells (22, 23, 27). However, other groups have shown no significant difference in apoptotic cell death between normal human CD4 and CD8 T-lymphocytes treated with gamma or proton radiation (28). Our results demonstrate that proton irradiation more effectively sensitizes NSCLC cells than gamma irradiation, suggesting that the effect of proton

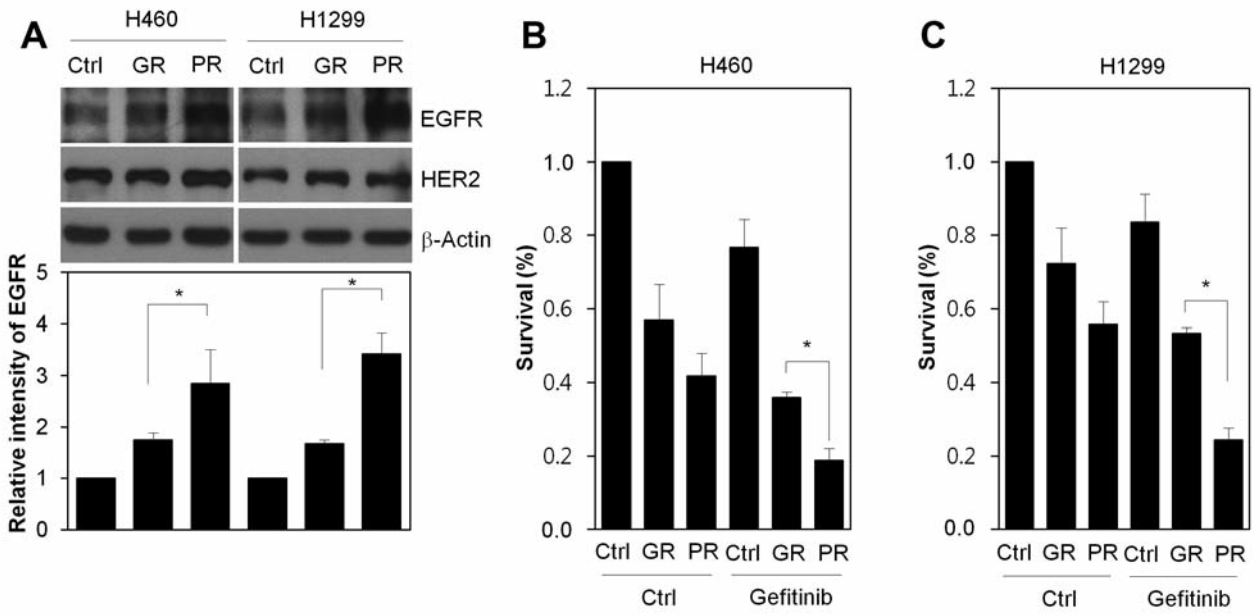

Figure 4. *Gefitinib preferentially sensitized non-small cell lung cancer cells to proton irradiation compared to gamma radiation. A: H460 and H1299 cells were treated with 6 Gy of proton (PR) or gamma irradiation (GR). The levels of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) in the cells were analyzed by immunoblotting with anti-EGFR and anti-HER2 antibodies (upper panel). β-Actin was used as a loading control. The intensities of EGFR expression were quantified by Image J from three independent experiments (lower panel). H460 (B) and H1299 (C) cells untreated (Ctrl) or treated with 3 Gy of GR or PR in the absence (Ctrl; dimethyl sulfoxide) or presence of 1 μM gefitinib. Clonogenic survival was assessed by colony-formation assay. Colony formation was quantified by an automatic colony counter. The data represent typical results and are presented as the mean±standard deviation of three independent experiments; *p<0.01 compared to gamma-irradiated cells.*

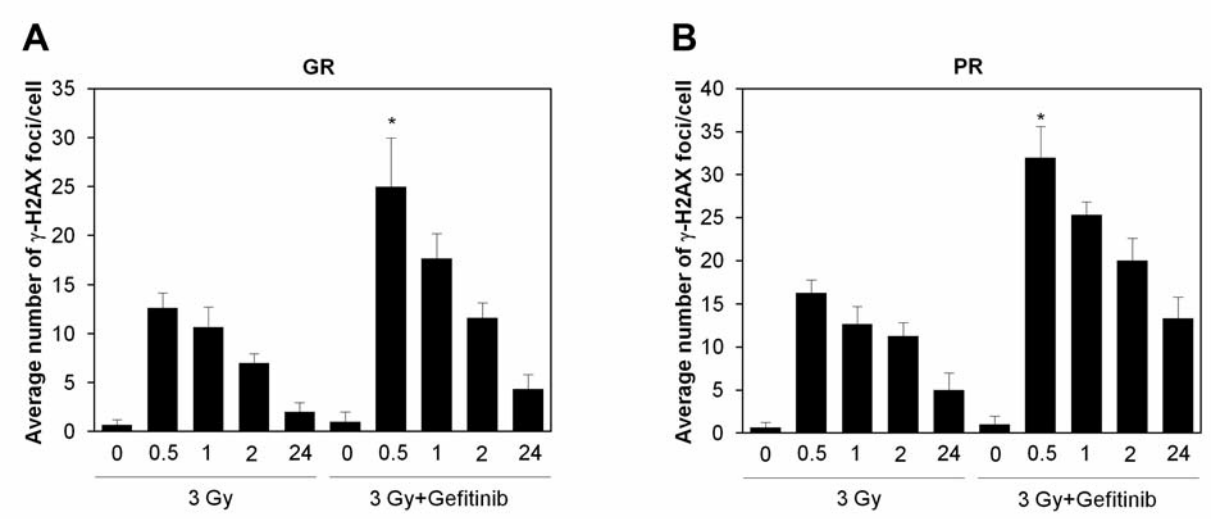

Figure 5. *Gefitinib suppressed the repair of radiation-induced DNA double-strand breaks as determined by the presence of γ-H2AX foci. H1299 cells were treated with 3 Gy of gamma (A; GR) or proton (B; PR) radiation in the absence (dimethyl sulfoxide) or presence of 1 μM gefitinib. After irradiation, the cells were fixed at the indicated time points for analysis of γ-H2AX foci using immunofluorescence confocal microscopy. The data are presented as the mean±standard deviation of three independent experiments. *p<0.01 compared to irradiated cells.*

radiation-induced apoptotic cell death is dependent on the cell type, such as cancer cells or normal cells. Regarding cell death, the size and number of γ-H2AX foci, indicative of DNA damage, significantly increase with proton treatment relative to gamma irradiation (22, 26). Similarly, our data also indicate that proton irradiation increased DNA damage

more than gamma irradiation did in H1299 cells. Therefore, it is possible that greater energy deposition by proton irradiation causes substantial DNA damage, leading to higher apoptotic cell death than does gamma irradiation. In addition, this may be the reason behind the differing sensitivities to proton irradiation depending on cell type.

The antibody array analysis showed that many signaling proteins, such as EGFR, were differentially regulated by proton irradiation compared to gamma irradiation. Interestingly, with regard to proteins, we also found that DNA fragmentation factor subunit alpha (DFFA) was specifically induced by proton irradiation (data not shown). Since DFFA is the substrate for caspase-3 and triggers DNA fragmentation during apoptosis (29), DFFA induction in proton-irradiated cells reflects the apoptotic phenotype and DNA damage observed with proton irradiation. Interestingly, we also observed that expression of the claudin family was significantly modulated by proton irradiation (Figure 3). Recent reports suggest that claudins, important components of tight junctions, are associated with tumorigenesis and chemoresistance (30). However, little is known on the role of the claudin family in radiotherapy, and it will be interesting to determine the role of claudins in proton radiotherapy.

Our study provides evidence that proton irradiation enhances EGFR expression and that inhibition of EGFR sensitizes radioresistant NSCLC cells by modulating DNA repair. Increased EGFR expression and activity are often correlated with tumor resistance to radiotherapy (13, 14). A recent study showed that EGFR modulates DNA repair after radiation-induced damage by associating with the catalytic subunit of DNA protein kinase (DNA-PKcs) (31). The nuclear localization and binding of DNA-PKcs to EGFR was induced by irradiation (11, 31). Furthermore, it is known that EGFR inhibitors counteract the nuclear translocation of DNA-PK subunits (11). Therefore, it appears that proton-mediated EGFR regulates nuclear DNA-PKcs activity, and consequently contributes to radioresistance of NSCLC cells. Paradoxically, the fact that proton irradiation more strongly induces EGFR than does gamma irradiation implies that proton irradiation induces both a potent cell-killing effect and a cytoprotective response as a negative feedback mechanism.

In summary, our work reveals signaling protein profiles of radioresistant and radiosensitive NSCLC cells following proton irradiation and demonstrates that proton-induced EGFR regulates the radioresistance of NSCLC cells by modulating DNA repair capacity. This study provides, to our knowledge, first evidence that proton irradiation can sensitize radioresistant NSCLC cells by regulating EGFR-mediated DNA repair.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by the Korean Health Technology R&D Project, Ministry of Health & Welfare (No. HI14C1864) and the National R&D Program through the Korea Institute of Radiological and Medical Sciences funded by the Ministry of Science, ICT & Future Planning (No. 1711021781).

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90, 2011.
2 Bentzen SM: Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. Nat Rev Cancer *6*: 702-713, 2006.
3 Berman AT, James SS, and Rengan R: Proton beam therapy for non-small cell lung cancer: Current clinical evidence and future directions. Cancers *7*: 1178-1190, 2015.
4 Girdhani S, Sachs R, and Hlatky L: Biological effects of proton radiation: what we know and don't know. Radiat Res *179*: 257-272, 2013.
5 Shaw RJ and Cantley LC: Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature *441*: 424-430, 2006.
6 Sharma SV, Bell DW, Settleman J, and Haber DA: Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer *7*: 169-181, 2007.
7 Okamoto I, Takahashi T, Okamoto H, Nakagawa K, Watanabe K, Nakamatsu K, Nishimura Y, Fukuoka M, and Yamamoto N: Single-agent gefitinib with concurrent radiotherapy for locally advanced non-small cell lung cancer harboring mutations of the epidermal growth factor receptor. Lung Cancer *72*: 199-204, 2011.
8 Tanaka T, Munshi A, Brooks C, Liu J, Hobbs ML and Meyn RE: Gefitinib radiosensitizes non-small cell lung cancer cells by suppressing cellular DNA repair capacity. Clin Cancer Res *14*: 1266-1273, 2008.
9 Rothschild S, Bucher SE, Bernier J, Aebersold DM, Zouhair A, Ries G, Lombrieser N, Lippuner T, Lutolf UM, Glanzmann C and Ciernik IF: Gefitinib in combination with irradiation with or without cisplatin in patients with inoperable stage III non-small cell lung cancer: a phase I trial. Int J Radiat Oncol Biol Phys *80*: 126-132, 2011.
10 Chi A, Remick S and Tse W: EGFR inhibition in non-small cell lung cancer: current evidence and future directions. Biomark Res *1*: 2, 2013.
11 Rodemann HP, Dittmann K and Toulany M: Radiation-induced EGFR-signaling and control of DNA-damage repair. Int J Radiat Biol *83*: 781-791, 2007.
12 Szumiel I: Epidermal growth factor receptor and DNA double-strand break repair: the cell's self-defence. Cell Signal *18*: 1537-1548, 2006.
13 Milas L, Fan Z, Andratschke NH and Ang KK: Epidermal growth factor receptor and tumor response to radiation: *in vivo* preclinical studies. Int J Radiat Oncol Biol Phys *58*: 966-971, 2004.
14 Riesterer O, Milas L and Ang KK: Use of molecular biomarkers for predicting the response to radiotherapy with or without chemotherapy. J Clin Oncol *25*: 4075-4083, 2007.
15 Kedracka-Krok S, Jankowska U, Elas M, Sowa U, Swakon J, Cierniak A, Olko P, Romanowska-Dixon B and Urbanska K: Proteomic analysis of proton beam irradiated human melanoma cells. PLoS One *9*: e84621, 2014.

16 Gridley DS, Freeman TL, Makinde AY, Wroe AJ, Luo-Owen X, Tian J, Mao XW, Rightnar S, Kennedy AR, Slater JM and Pecaut MJ: Comparison of proton and electron radiation effects on biological responses in liver, spleen and blood. Int J Radiat Biol *87*: 1173-1181, 2011.

17 Finnberg N, Wambi C, Ware JH, Kennedy AR and El-Deiry WS: Gamma-radiation (GR) triggers a unique gene expression profile associated with cell death compared to proton radiation (PR) in mice *in vivo*. Cancer Biol Ther *7*: 2023-2033, 2008.

18 Ha BG, Park JE, Cho HJ, Lim YB and Shon YH: Inhibitory effects of proton beam irradiation on integrin expression and signaling pathway in human colon carcinoma HT29 cells. Int J Oncol *46*: 2621-2628, 2015.

19 Kim JS, Chang JW, Yun HS, Yang KM, Hong EH, Kim DH, Um HD, Lee KH, Lee SJ and Hwang SG: Chloride intracellular channel 1 identified using proteomic analysis plays an important role in the radiosensitivity of HEp-2 cells *via* reactive oxygen species production. Proteomics *10*: 2589-2604, 2010.

20 Choe MH, Min JW, Jeon HB, Cho DH, Oh JS, Lee HG, Hwang SG, An S, Han YH and Kim JS: ERp57 modulates STAT3 activity in radioresistant laryngeal cancer cells and serves as a prognostic marker for laryngeal cancer. Oncotarget *6*: 2654-2666, 2015.

21 Kim JS, Kim EJ, Oh JS, Park IC and Hwang SG: CIP2A modulates cell-cycle progression in human cancer cells by regulating the stability and activity of Plk1. Cancer Res *73*: 6667-6678, 2013.

22 Gerelchuluun A, Hong Z, Sun L, Suzuki K, Terunuma T, Yasuoka K, Sakae T, Moritake T and Tsuboi K: Induction of *in situ* DNA double-strand breaks and apoptosis by 200 MeV protons and 10 MV X-rays in human tumour cell lines. Int J Radiat Biol *87*: 57-70, 2011.

23 Di Pietro C, Piro S, Tabbi G, Ragusa M, Di Pietro V, Zimmitti V, Cuda F, Anello M, Consoli U, Salinaro ET, Caruso M, Vancheri C, Crimi N, Sabini MG, Cirrone GA, Raffaele L, Privitera G, Pulvirenti A, Giugno R, Ferro A, Cuttone G, Lo Nigro S, Purrello R, Purrello F and Purrello M: Cellular and molecular effects of protons: apoptosis induction and potential implications for cancer therapy. Apoptosis *11*: 57-66, 2006.

24 Kim MJ, Yun HS, Hong EH, Lee SJ, Baek JH, Lee CW, Yim JH, Kim JS, Park JK, Um HD and Hwang SG: Depletion of end-binding protein 1 (EB1) promotes apoptosis of human non-small-cell lung cancer cells *via* reactive oxygen species and Bax-mediated mitochondrial dysfunction. Cancer Lett *339*: 15-24, 2013.

25 Girdhani S, Lamont C, Hahnfeldt P, Abdollahi A and Hlatky L: Proton irradiation suppresses angiogenic genes and impairs cell invasion and tumor growth. Radiat Res *178*: 33-45, 2012.

26 Leatherbarrow EL, Harper JV, Cucinotta FA and O'Neill P: Induction and quantification of gamma-H2AX foci following low and high LET-irradiation. Int J Radiat Biol *82*: 111-118, 2006.

27 Green LM, Tran DT, Murray DK, Rightnar SS, Todd S and Nelson GA: Response of thyroid follicular cells to gamma irradiation compared to proton irradiation: II. The role of connexin 32. Radiat Res *158*: 475-485, 2002.

28 Radojcic M and Crompton NE: Age dependence of T-lymphocyte apoptosis induced by high-energy proton exposure. Radiat Environ Biophys *40*: 131-135, 2001.

29 Liu X, Zou H, Slaughter C and Wang X: DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell *89*: 175-184, 1997.

30 Morin PJ: Claudin proteins in human cancer: promising new targets for diagnosis and therapy. Cancer Res *65*: 9603-9606, 2005.

31 Liccardi G, Hartley JA and Hochhauser D: EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment. Cancer Res *71*: 1103-1114, 2011.

Received November 6, 2015
Revised December 7, 2015
Accepted December 14, 2015