의학박사 학위논문

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이 논문을 의학박사 학위논문으로 제출함
2013년 10월

서울대학교 대학원
의학과 방사선종양학 전공
김 규 보

김규보의 의학박사 학위논문을 인준함
2014년 1월

위 원 장 _____ (인)
부위원장 _____ (인)
위 원 _____ (인)
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ABSTRACT

Purpose: The purpose of this study is to suggest the potential mechanisms of epidermal growth factor (EGF)-induced cell death and radiosensitization in epidermal growth factor receptor (EGFR)-overexpressing cancer cell lines.

Methods: Three EGFR-overexpressing cancer cell lines (AMC-HN3, A431, and SQ20B), one EGFR-null cancer cell line (H520) and normal fibroblasts were cultured with 0.01-1000nM of recombinant human EGF (rhEGF), and clonogenic assays were performed. After culturing serum-starved cells with 10 nM rhEGF, the expression patterns of two apoptosis-associated proteins (cleaved caspase-3 and cleaved PARP) and the PI3K/Akt/mTOR signaling pathway were measured using immunoblotting. EGFR and K-ras mutation analyses were performed. In addition, EGFR phosphorylation and p38 expression after rhEGF treatment were measured. The radiosensitizing effect of EGF was also evaluated with a clonogenic assay and γH2AX immunofluorescence.

Results: In the clonogenic assay, the number of colonies was decreased in a dose-dependent manner in EGFR-overexpressing cell lines, whereas rhEGF treatment increased the number of colonies in the normal fibroblast, and there was no change in the number of colonies in the EGFR-null cell line. Significant expression of cleaved

caspase-3 and cleaved PARP was induced in EGFR-overexpressing cell lines, whereas there was no expression in the normal fibroblast, and no expression of cleaved caspase-3 or significant temporal increase in the expression of cleaved PARP in EGFR-null cell line. As for the PI3K/Akt/mTOR signaling pathway, rhEGF treatment suppressed the expression of PI3K, Akt, and mTOR in a time-dependent manner in EGFR-overexpressing cell lines. K-ras mutations were not detected on any of the cancer cell lines, whereas exon 20 missense substitution mutation of the EGFR gene was detected on A431. rhEGF treatment induced phosphorylation of EGFR residue Tyr845 in normal fibroblast, whereas EGFR was phosphorylated at Tyr845, Tyr992, Tyr1045 and Tyr1068 in AMC-HN3 and A431 treated with rhEGF. However, there was no change in the p38 expression in AMC-HN3 and A431 after rhEGF treatment. As for the radiosensitizing effect, EGF enhanced the radiosensitivity of AMC-HN3 and A431 cells, and increased radiation-induced γH2AX foci formation.

Conclusion: rhEGF treatment induced cell death in EGFR-overexpressing cancer cell lines, and the mode of cell death was apoptosis. And, the cell death may be associated with the paradoxical suppression of the PI3K/Akt/mTOR signaling pathway. rhEGF in combination with radiation augmented the radiation effect in EGFR-overexpressing cancer cell lines via inhibition of DNA damage repair.

Key words: EGF, EGFR-overexpressing cancer cell line, apoptosis, PI3K-Akt-mTOR signaling pathway, radiosensitization

Student number: 2004-30600

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INTRODUCTION

Epidermal growth factor (EGF) was isolated in the early 1960's, and was named as such because it stimulates the proliferation of epithelial cells (1). It is produced by platelets, macrophages, and monocytes (2), and is found in various normal tissues and body fluids such as skin, mucosa, tears, and saliva. EGF is well known to interact with the EGF receptor (EGFR) on epidermal cells and fibroblasts, and to induce epithelial proliferation, modification of migration, and differentiation processes (3,4).

In the meanwhile, EGF-induced cancer cell death in several EGFR-overexpressing cancer cell lines has been reported as early as the 1980's (5,6), with the mode of cell death known to be apoptosis. We confirmed EGF-induced apoptosis in EGFR-overexpressing cancer cell lines. When combined with radiation, EGF does not inhibit the cell killing effect of radiation and even enhances the radiation-induced cell death in EGFR-overexpressing cancer cell lines (7,8). However, the exact mechanism of EGF-induced cancer cell death and radiosensitization has yet to be elucidated.

The main pathways downstream of EGFR activation include PI3K/Akt/mTOR pathway, RAS/MAPK pathway, PLCγ/PKC pathway, and STATs pathway (9). Among these, STATs pathway is the most intensively investigated mechanism involving EGF-induced cancer cell death (10). However, the consequences of other signal pathway

activation are still unclear. Moreover, PI3K/Akt/mTOR pathway activation is generally considered to enhance tumor growth, therefore, the EGF-induced cancer cell death is difficult to be explained related to this pathway.

In this study, we evaluated the changes of expression of the PI3K/Akt/mTOR signaling pathway after EGF treatment in EGFR-overexpressing cancer cell lines to suggest the potential mechanisms of EGF-induced cancer cell death. In addition, we also evaluated the radiosensitizing effect of EGF and its mechanism.

MATERIALS AND METHODS

Study drug

rhEGF used in this study was provided by Daewoong Pharmaceutical Co. Ltd. (Seoul, Korea). Spray form of this product is commercially available and prescribed for diabetic foot in Korea.

Cell lines

A431 (human epidermoid carcinoma) and H520 (human lung squamous cell carcinoma) were purchased from the American Type Culture Collection (ATCC). AMC-HN3 (human head and neck squamous cell carcinoma) and SQ20B (human laryngeal squamous cell carcinoma) were generous gifts from Prof. Sang-wook Lee at University of Ulsan, Seoul, Korea, and Prof. In-Ah Kim at Seoul National University, Seoul, Korea, respectively. Normal primary human cervix fibroblast was also used, and this was a kind gift from Prof. Yong-Sang Song at Seoul National University, Seoul, Korea. Cells were kept at 37℃ and 5% CO_2 and cultured in RPMI 1640 (Gibco BRL, Gaithersburg, MD, USA), DMEM (Gibco BRL, 31600 034, Grand Island, NY, USA), and MEM (Gibco BRL). All media were supplemented with 10% FBS (Sigma, St. Louis, MO, USA). Serum-free media (0.05% FBS) were also used in order to exclude the effects of other growth factors in the FBS.

In vitro clonogenic assay: rhEGF alone

Mid-log phase cells from monolayer cultures were trypsinized and plated in six-well culture plates at cell densities that were optimized for the experimental conditions indicated: 2 x 102 cells were plated for AMC-HN3 cells, and 1 x 102 cells for other cell lines according to treatment conditions.

The cells were then incubated for 24 hours prior to treatment. To measure the effects of rhEGF, 0, 0.1, 1, 10, 100, and 1000 nM rhEGF were added to the medium. Cells were incubated for an additional 10 days at 37 °C without changing the medium, resulting in the formation of colonies.

The cells were then fixed with 100% methanol and stained with 0.5% crystal violet in methanol. The numbers of colonies (defined as more than 50 cells) were counted. Surviving fraction (SF) was defined as the ratio of the numbers of colonies to the numbers of initially plated cells corrected by plating efficiency (PE), where PE was the SF of untreated cells. Each experiment was independently repeated three times.

Western blot analysis

Cells were harvested using cold lysis buffer (iNtRON Biotechnology; Seoul, Korea). The lysates were sonicated, clarified by centrifugation at 13000 rpm for 20 minutes at 4 °C, and the supernatants were utilized for analysis. Protein concentrations of the samples were

determined with a BCA protein assay kit (PIERCE; Rockford, IL, USA.). The protein was mixed with 5X sample buffer (2% SDS, 5% β-mercaptoethanol, 20% glycerol, 0.001% bromophenol-blue, 0.1 M Tris-HCl, pH 6.8) and boiled for 10 minutes. Equal amounts of protein were separated by 8-12% SDS-PAGE and transferred to PVDF membranes (Millipore Corp; Bedford, MA, USA). Membranes were blocked for 1 hour at room temperature with TBST (10 nM Tris-HCl [pH 7.5], 150 mM NaCl, 0.1% Tween 20) containing 5% dry milk, and then washed three times with TBST. Membranes were probed at 4 °C overnight with polyclonal rabbit anti-EGFR (Cell Signaling Technology; Beverly, MA, USA), pEGFR (Cell Signaling Technology), cleaved caspase-3 (Cell Signaling Technology), cleaved PARP (Cell Signaling Technology), AKT (Cell Signaling Technology), PI3K (Cell Signaling Technology), mTOR (Cell Signaling Technology), p38 (Cell Signaling Technology), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). The dilutions were 1:500 for anti-EGFR, and 1:1000 for other antibodies. The blots were then incubated with peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories; West Grove, PA, USA) at a 1:1000 dilution for 2 hours at room temperature and washed three times with TBST. As a loading control, the same membranes were stripped and probed with monoclonal anti-α-tubulin (Sigma) at a 1:3,000 dilution at 4 °C overnight, followed by an incubation with the goat anti-mouse IgG used above at a 1:1000 dilution for 1 hour at room temperature and three washes with TBST. Immunoreactive

proteins were visualized with WEST-ONE TM chemiluminescent substrate (iNtRON Biotechnology) and signals were detected with LAS-3000 (FujiFilm, Tokyo, JAPAN).

EGFR and K-ras mutation analysis

EGFR and K-ras mutation analysis was performed using direct sequencing. All sequencing reactions were performed in both forward and reverse directions, and the electropherograms were analyzed. For EGFR mutation, exons 18, 19, 20, and 21 of the EGFR genes were screened. The primers for PCR amplification of EGFR genes were as follows: exon 18, forward: 5'- tccaaatgagctggcaagtg, reverse: 5'- tcccaaacactcagtgaaacaaa; exon 19, forward: 5'- atgtggcaccatctcacaattgcc, reverse: 5'- ccacacagcaaagcagaaactcac; exon 20, forward:5'- cattcatgcgtcttcacctg, reverse: 5'- catatccccatggcaaactc; exon 21, forward: 5'- gctcagagcctggcatgaa, reverse: 5'- catcctcccctgcatgtgt. And for K-ras mutation, exon 1 codons 12, 13 and exon 2 codon 61 were screened. The primers for PCR amplification of K-ras genes were as follows: exon 1, codons 12 and 13, forward: 5'-ttatgtgtgacatgttctaat, reverse: 5'-agaatggtcctgcaccagtaa; exon 2, codon 61, forward: 5'-tcaagtcctttgcccatttt, reverse: 5'-tgcatggcattagcaaagac.

In vitro clonogenic assay: combination of rhEGF and

radiation

In the experiments using radiation with or without rhEGF, 1 x 10^2 cells were plated for doses of 2 Gy, 3 x 10^2 cells for 5 Gy, and 1 x 10^3 cells for 10 Gy, for all cancer cell lines (AMC-HN3, A431, SG20B, and H520). The cells were then incubated for 24 hours prior to treatment. To compare the combined effect of rhEGF and radiation with that of radiation alone, cells were irradiated with 6 MV X-rays generated by a linear accelerator (Clinac 6EX, Varian, Palo Alto, CA), at doses of 0, 2, 5, and 10 Gy. Where indicated, rhEGF was added to the media at a concentration of 10 nM for 24 hours, after which cells were irradiated in the presence of rhEGF. After 24 hours, the medium was removed, and cells were incubated in drug-free media for 10 days to form colonies. Each experiment was independently repeated three times.

Immunofluorescent detection of γH2AX

Cells were grown and treated in tissue culture chamber slides (Nalge Nunc International, Naperville, IL). At the specified times, the medium was aspirated and cells were fixed in 4% paraformaldehyde for 10 minutes at room temperature. The paraformaldehyde was aspirated, and the cells were treated with a 0.2% NP40/PBS solution for 15 minutes. The cells were then washed in PBS twice, and the anti-γH2AX antibody (Cell Signaling Technology) was added at a dilution of 1:200 in 1% bovine serum albumin and incubated overnight at 4C. The

cells were again washed twice in PBS before incubating in the dark with an FITC-labeled secondary antibody (Invitrogen, Camarillo, CA) at a dilution of 1:50 in 1% bovine serum albumin for 1 hour. The secondary antibody solution was then aspirated and the cells were washed twice in PBS. The cells were then incubated in the dark with 4,6-diamindino-2-phenylindole (1µg/mL) in PBS for 30 minutes and washed twice, and coverslips were mounted with an antifade solution (Vector Laboratories, Burlingame, CA). Slides were examined on a Leica DMRXA fluorescent microscope (Leica, Wetzlar, Germany). Images were captured by a Photometrics Sensys CCD camera (Leica, Wetzlar, Germany).

RESULTS

Effect on cell proliferation

AMC-HN3, A431, SQ20B, H520, and normal fibroblasts were cultured with varying concentrations of rhEGF (0.01-1000nM) for five days, and clonogenic assays were performed. For AMC-HN3, A431, and SQ20B, the number of colonies was decreased in a dose-dependent manner, whereas the number of colonies was increased in normal fibroblast. For H520, which is the EGFR-null cell line, there was no change in the number of colonies. (Figure 1)

Effect on apoptosis-associated proteins

After culturing serum-starved cells with 10 nM rhEGF for varying time periods (5 min, 4 hr, 24 hr, 48 hr, and 72 hr), we measured the expression levels of two apoptosis-associated proteins, cleaved caspase-3, and cleaved PARP,using immunoblotting .

Treatment with rhEGF induced a significant temporal increase of cleaved caspase-3 and cleaved PARP expression in the EGFR-overexpressing cell lines (AMC-HN3, A431, and SG20B), whereas there was no expression in normal fibroblast. For H520, there was no expression of cleaved caspase-3 and no significant temporal increase in the expression of cleaved PARP. (Figure 2)

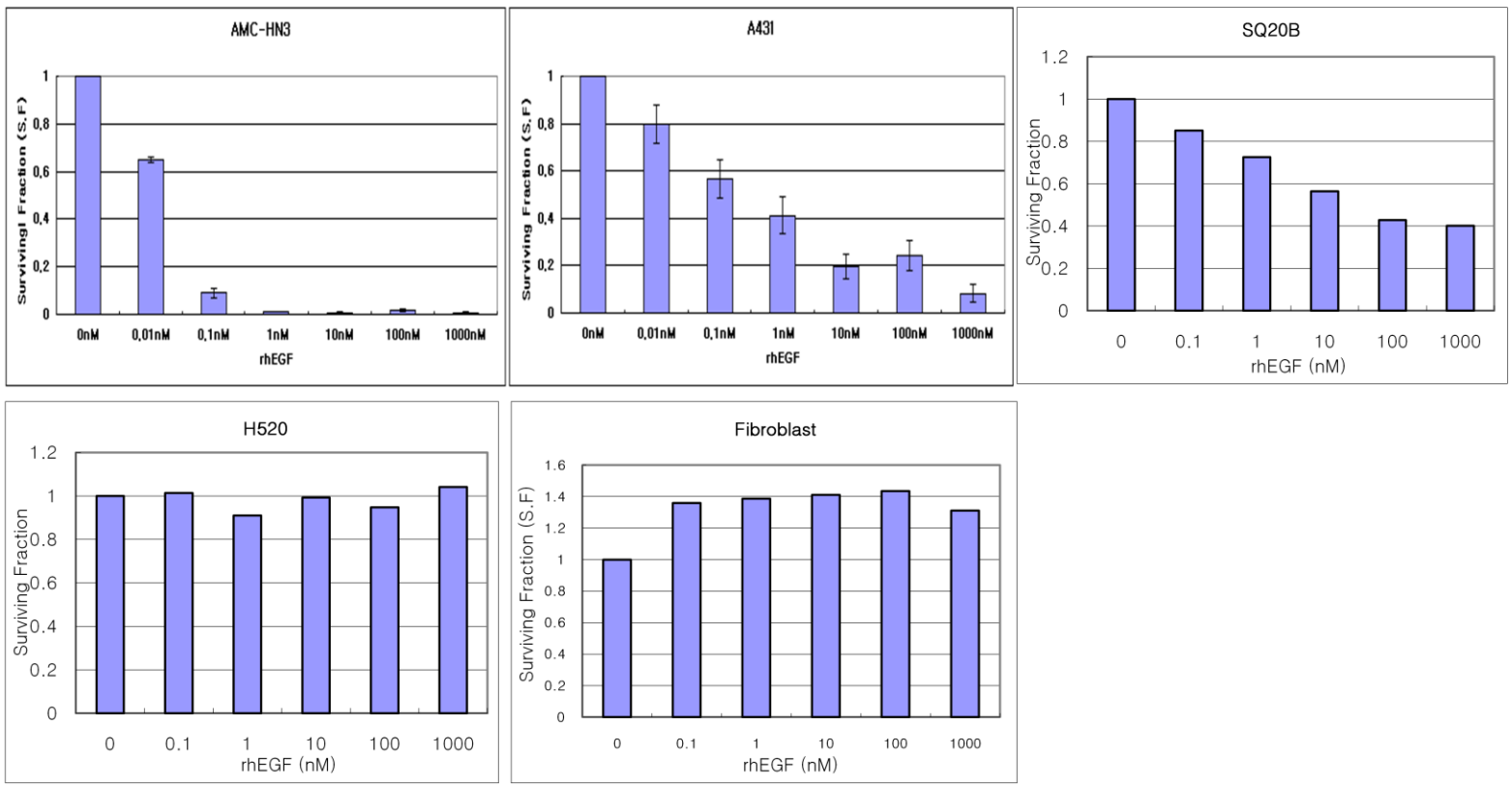


Figure 1. Clonogenic assays of rhEGF-treated cells

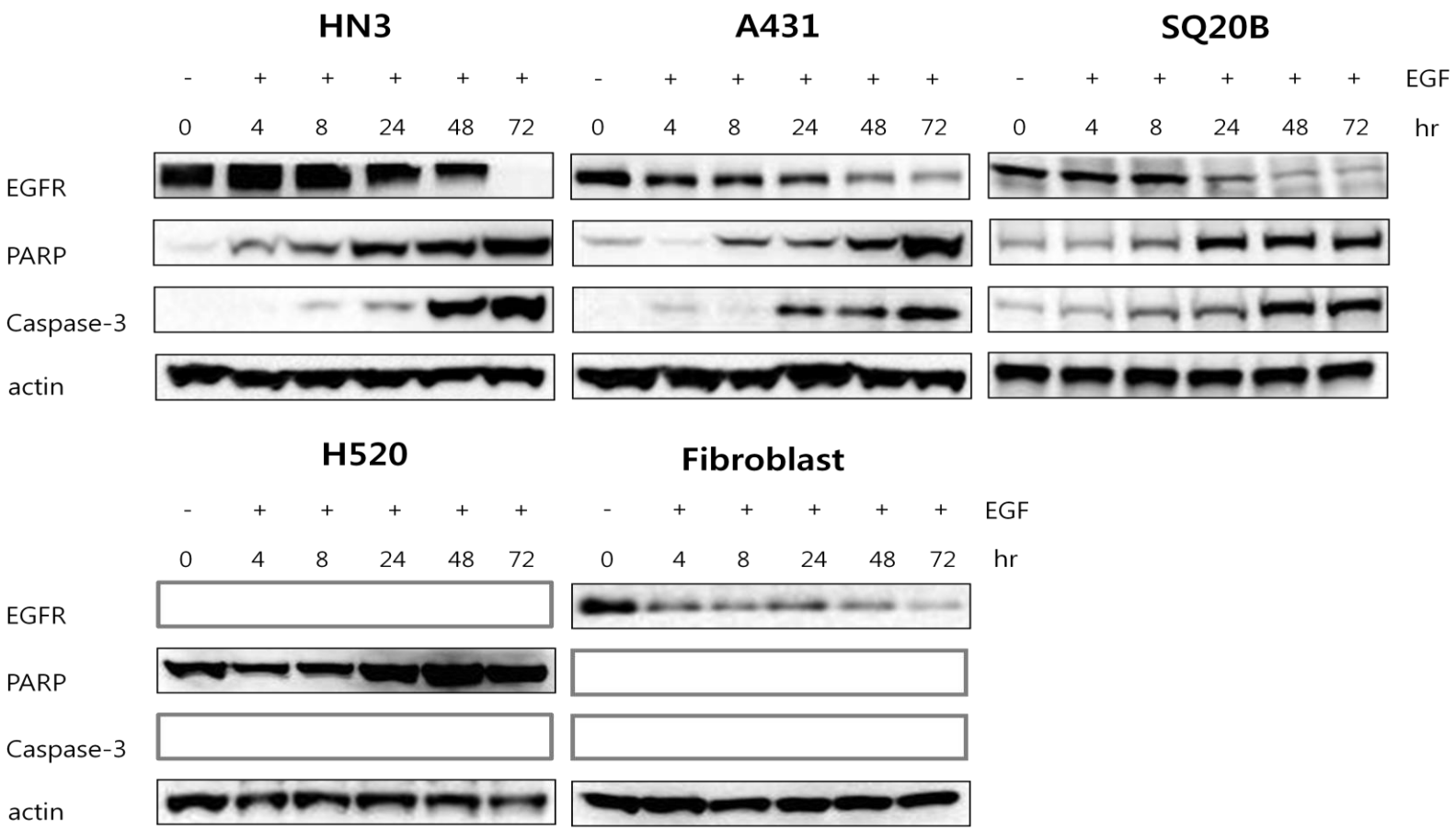


Figure 2. rhEGF-induced changes in expression of EGFR, PARP, and caspase-3 with respect to time

Effect on PI3K/Akt/mTOR signaling pathway

PI3K/Akt/mTOR pathways including PI3K, Akt, and mTOR were evaluated in a similar manner as above.

rhEGF was not associated with significant increase in the expression of PI3K, Akt, and mTOR in EGFR-overexpressing cell lines (AMC-HN3, A431, and SG20B), whereas Akt and mTOR expression was substantially enhanced in H520 and normal fibroblast. (Figure 3)

EGFR and K-ras mutation analysis

K-ras mutation was not detected on any of the cancer cell lines. However, exon 20 missense substitution mutation of the EGFR gene was detected on the A431 cell line. (Figure 4)

Effect on EGFR phosphorylation

To further elucidate the mechanism of EGF-induced cancer cell death, we measured the EGFR phosphorylation after culturing serum-starved cells with 10 nM rhEGF for varying time periods (5 min, 10min, 30min and 60 min) in the two EGFR-overexpressing cancer cell lines (AMC-HN3 and A431) and normal fibroblast.

rhEGF treatment induced phosphorylation of EGFR residue Tyr845 in normal fibroblast, whereas EGFR was phosphorylated at Tyr845, Tyr992, Tyr1045 and Tyr1068 in AMC-HN3 and A431 cell lines treated with rhEGF. (Figure 5)

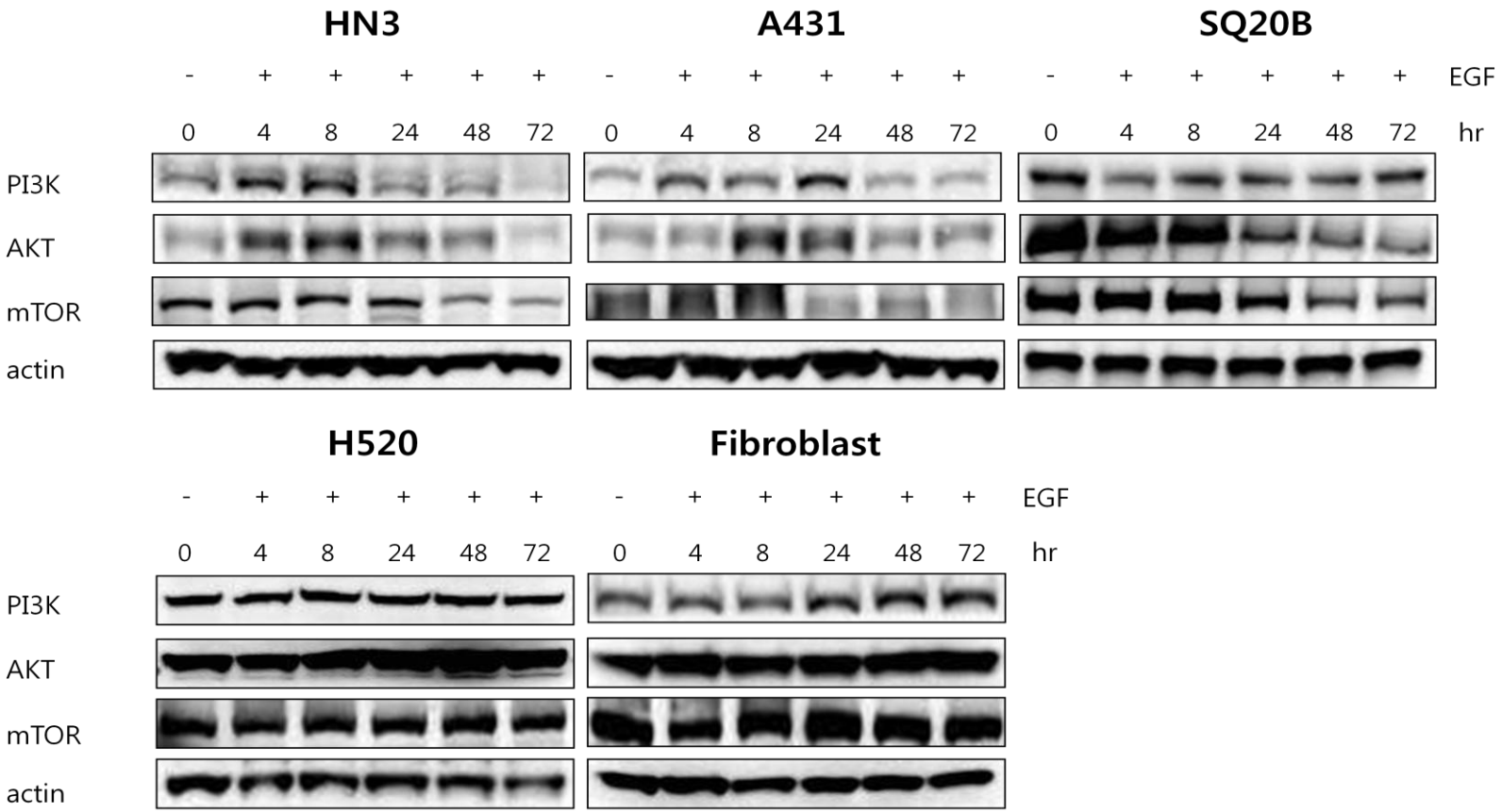


Figure 3. rhEGF-induced changes in expression of PI3K/Akt/mTOR signaling pathway with respect to time

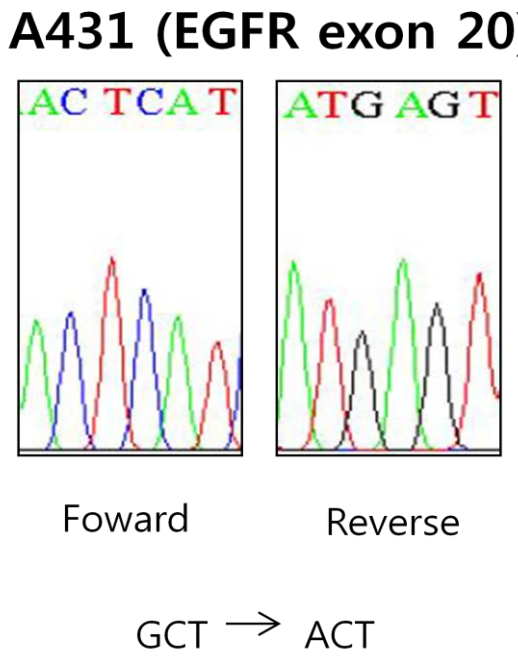


Figure 4. EGFR mutation analysis using direct sequencing for A431

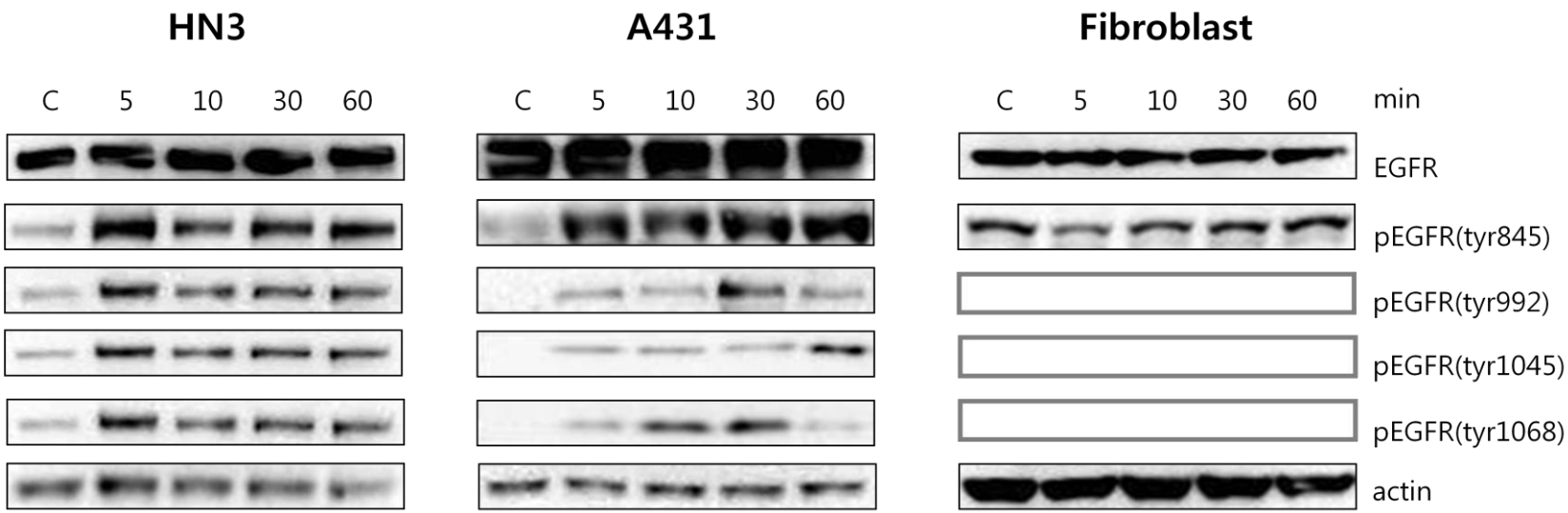


Figure 5. rhEGF-induced changes in EGFR phosphorylation

Effect on p38 expression

We also measured the p38 expression after rhEGF treatment with or without radiation. There was no change in the p38 expression regardless of the presence of rhEGF and/or radiation, nor did the radiation dose have an effect. (Figure 6)

Combination of rhEGF and radiation

We subjected two EGFR-overexpressing cell lines (AMC-HN3 and A431) to ionizing radiation, followed by a ten-day culture with either an unsupplemented medium or a medium containing 10nM of rhEGF. In the clonogenic assays, we observed that rhEGF had a radio-sensitizing effect on both cell lines. (Figure 7)

PI3K/Akt/mTOR pathways were also evaluated in the experiment combining rhEGF and radiation. There was no significant increase in the expression of PI3K, Akt, or mTOR in either of the cell lines. (Figure 8)

Immunofluorescent detection of γH2AX

The radiosensitizing effect of EGF was assessed by γH2AX immunofluorescence. (Figure 9) Radiation-induced γH2AX foci formation in AMC-HN3 (B and C), A431 (E and F), and normal fibroblast (H and I) cells was shown. For comparison, cells treated with 5 Gy alone (B, E, H), and cells exposed to 10nM rhEGF prior to 5 Gy

irradiation (C, F, I) are shown. Cells were fixed and stained for γH2AX analysis 2 hours after irradiation.

In both AMC-HN3 and A431 cells, the immunofluorescence visualization of nuclei and γH2AX foci were detected. Cells treated with 5 Gy alone and cells exposed to 10nM rhEGF prior to 5 Gy irradiation were compared and increased radiation-induced γH2AX foci formation was found.

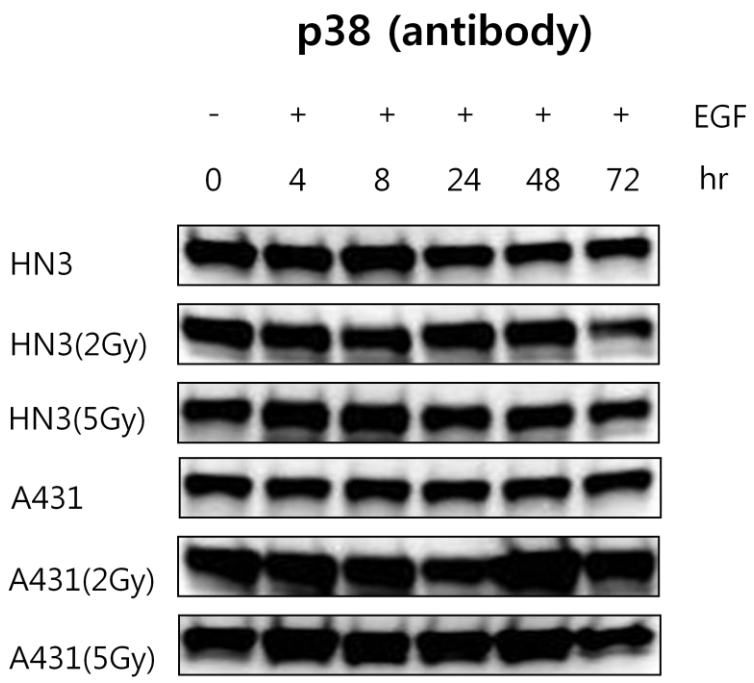
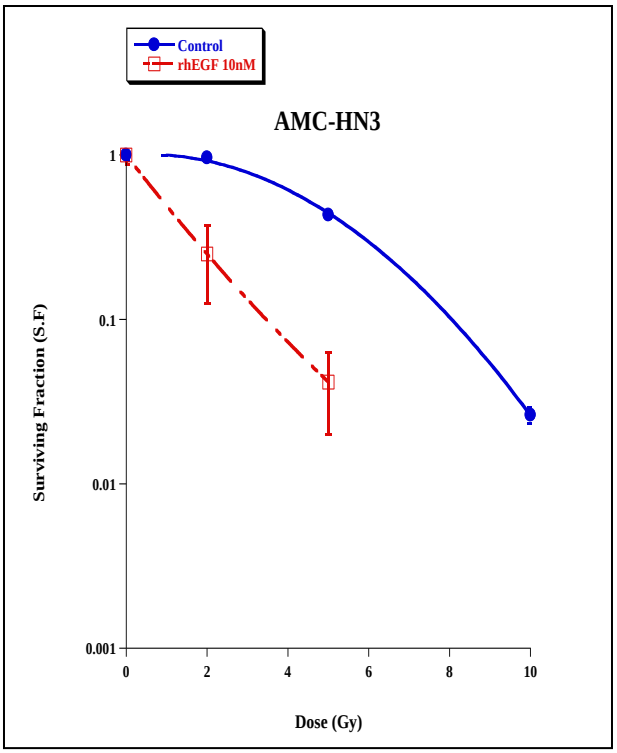


Figure 6. rhEGF and radiation-induced changes in p38 expression with respect to time



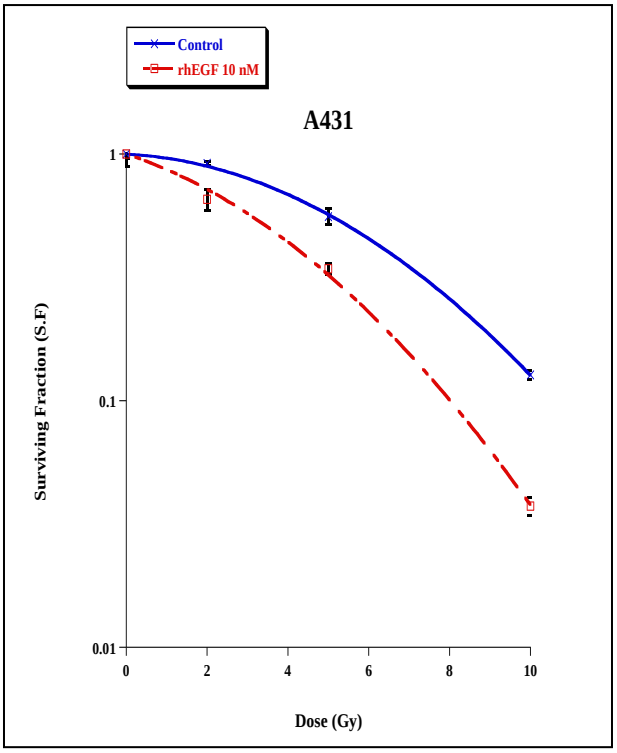


Figure 7. Effects of rhEGF on cell killing of ionizing radiation

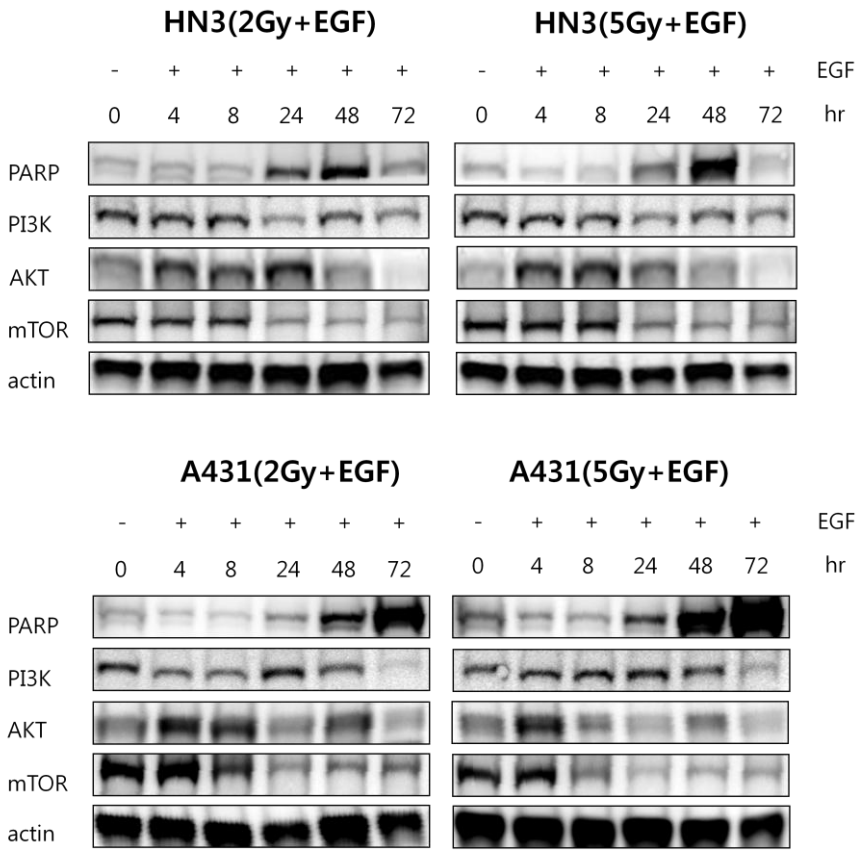


Figure 8. rhEGF and radiation-induced changes in expression of PI3K/Akt/mTOR signaling pathway with respect to time

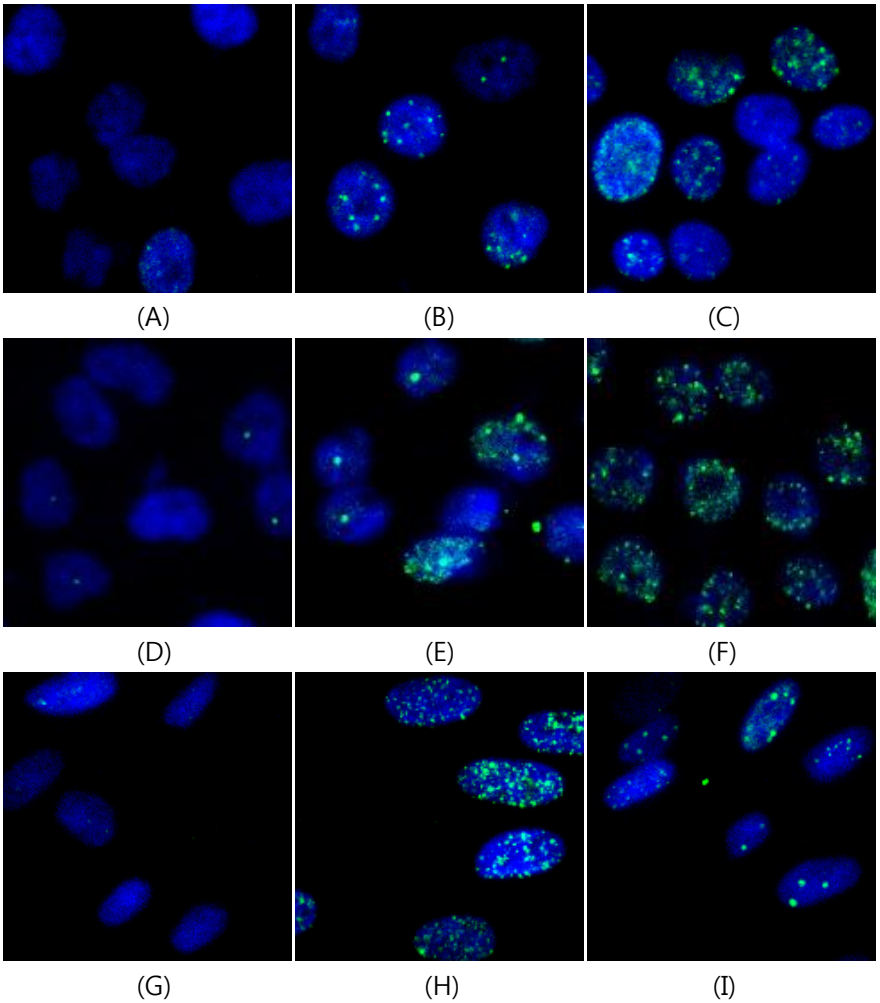


Figure 9. Immunofluorescent detection of γH2AX

(A) HN3, control (B) HN3, 5 Gy (C) HN3, 5 Gy + 10nM rhEGF

(D) A431, control (E) A431, 5 Gy (F) A431, 5 Gy + 10nM rhEGF

(G) fibroblast, control (H) fibroblast, 5 Gy (I) fibroblast, 5 Gy + 10nM rhEGF

DISCUSSION

EGFR is overexpressed in a number of cancers such as lung, stomach, head and neck, and others. The overexpression of EGFR is known to be an adverse prognosticator in these cancers (11-13). Therefore, the treatment strategy for these cancers has been focused on suppressing EGFR with the use of EGFR inhibitors such as tyrosine kinase inhibitor or monoclonal antibody. In contrast, EGF itself, one of the ligands activating EGFR, can cause cell death in EGFR-overexpressing cancer cell lines (5-8). EGF also enhances the radiosensitivity of EGFR-overexpressing cell lines when EGF is combined with radiation (6,7).

In this study, we reaffirmed that EGF induces apoptosis in several EGFR-overexpressing cancer cell lines, but not in EGFR-null cell line or normal fibroblast. Given that the growth inhibitory effect of EGF has been known to be associated with the concentration of EGF, we tested various concentrations of rhEGF, and demonstrated that concentrations as low as 0.01nM can cause significant cell death especially in AMC-HN3, moreover, a dose-response relationship was also observed in all of the EGFR-overexpressing cell lines. This finding was somewhat contradictory to that of Gulli's study, in which they demonstrated that 10nM EGF inhibited proliferation of A431 cells, but 0.01nM EGF increased cell proliferation compared to untreated cells (14). Given these observations, we used a 10nM dose of rhEGF in the subsequent experiments. In the immunoblotting,

the expression of cleaved caspase-3 and cleaved PARP was increased with time after rhEGF treatment, suggesting that the mechanism of EGF-induced cell death is apoptosis. Furthermore, the radiosensitizing effect of EGF in EGFR-overexpressing cell lines was also demonstrated.

As for the mechanism of EGF-induced cancer cell death, Chin et al. reported that STAT1 was activated by EGF, and then the activated STAT1 signaling pathway resulted in the expression of caspase 1 and apoptosis (15). Later, Grudinkin et al. also reported that EGF-induced apoptosis in A431 is dependent on STAT1, but not on STAT3 (16). More recently, Tikhomirov et al. (17) and Song et al. (18) suggested p38 MAPK-dependent apoptosis as the mechanism. In the present study, however, p38 expression was not associated with either the rhEGF treatment or radiation in the two EGFR-overexpressing cell lines (AMC-HN3 and A431).

It was the PI3K/Akt/mTOR signaling pathway that was found to be associated with cancer cell survival among the major pathways downstream of EGFR activation (19). In this study, we evaluated the changes of expression of the EGF-EGFR signaling pathway after rhEGF treatment in EGFR-overexpressing cancer cell lines. The paradoxical suppression of PI3K/Akt/mTOR signaling pathway was observed, which was contradictory to the expected observation with the activation of EGFR by EGF. This phenomenon was also observed when the two EGFR-overexpressing cell lines (AMC-HN3 and A431) were treated with rhEGF in combination with

radiation. However, the exact mechanism of the paradoxical suppression of PI3K/Akt/mTOR signaling pathway was not evaluated in our experiment. The initial activation of the pathway and the subsequent suppression could be interpreted as the feedback inhibition of the signaling pathway. However, the different patterns of EGFR phosphorylation between cancer cell lines and normal cervix fibroblast might suggest that the initial step of the EGF-EGFR signaling pathway is responsible for the deferential effect of rhEGF. One possible hypothesis is that the specific phospho-tyrosines serve to recruit specific effector molecules to activate several signaling pathways and the subsequent feedback inhibition. Further studies are needed for the elucidation of the exact mechanism.

Because it is well known that the efficacy of EGFR inhibitor is associated with the EGFR and/or K-ras mutation (20,21), we performed the mutation analysis for EGFR and K-ras. K-ras mutation was not detected on any of the cancer cell lines, while the EGFR mutation was detected on A431. However, the paradoxical suppression of PI3K/Akt/mTOR signaling pathway was observed on all of the EGFR-overexpressing cancer cell lines. Therefore, the EGF-induced cell death was considered to be independent of the EGFR mutation. Considering the EGFR tyrosine kinase inhibitor was effective mainly in patients with EGFR mutation, the anti-cancer effect of EGF may have clinical implications in the treatment of cancers without EGFR mutation. Song et al. already reported that EGF may be a more potent cytotoxic agent

than EGFR inhibitors (including cetuximab or gefitinib) in the EGFR-overexpressing cancer cell lines without EGFR mutation (18).

In conclusion, rhEGF treatment induced cell death in the EGFR-overexpressing cancer cell lines, and the mode of cell death was apoptosis. And, the cell death may be associated with the paradoxical suppression of PI3K/Akt/mTOR signaling pathway. EGF in combination with radiation augmented the radiation effect in EGFR-overexpressing cancer cell lines via inhibition of DNA damage repair.

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국문초록

목적: 본 연구의 목적은 상피세포 성장인자 수용체(EGFR)가 과발현된 암세포주에서 상피세포 성장인자 (EGF)에 의한 세포 사멸 효과와 방사선민감도 증가 기전을 규명하는 것이다.

방법: 3개의 EGFR 과발현 암세포주 (AMC-HN3, A431, SQ20B), EGFR이 발현되지 않는 암세포주 (H520)와 정상 섬유모세포를 사용하였고, 0.01-1000nM의 재조합 인간 상피세포 성장인자 (recombinant human EGF, rhEGF)를 처리한 후 세포집락 측정기법 (clonogenic assay)으로 생존 분획을 측정하였다. 10 nM rhEGF를 처리한 다음, 세포고사와 관련된 2개의 단백질 (cleaved caspase-3, cleaved PARP)과 PI3K, Akt 및 mTOR 발현을 웨스턴 블롯을 이용하여 측정하였다. EGFR과 K-ras 돌연변이 분석을 시행하였고, rhEGF 처리 후 EGFR 인산화 및 p38 발현을 측정하였다. rhEGF에 의한 방사선 감작 효과를 세포집락 측정기법을 통해 측정하고, γH2AX를 측정하여 DNA 손상 수복을 평가하였다.

결과: rhEGF를 처리한 후의 세포집락은, EGFR 과발현 세포에서는 rhEGF 농도가 증가할수록 감소하고, 정상 섬유모세포에서는 증가하며, EGFR 무발현 세포에서는 변화가 없었다. cleaved caspase-3와

cleaved PARP는, EGFR 과발현 세포에서는 rhEGF 처리 후 그 발현이 점차 증가하고, 정상 섬유모세포에서는 발현되지 않았으며, EGFR 무발현 세포에서는 cleaved caspase-3는 발현되지 않았고 cleaved PARP는 시간에 따른 증가 양상이 관찰되지 않았다. EGFR 과발현 세포에서는 rhEGF 처리에 의해 PI3K, Akt, mTOR의 발현이 억제되었다. K-ras 돌연변이는 모든 암세포주에서 없었고, EGFR 돌연변이는 A431 세포주에서 exon 20의 missense substitution mutation이 있었다. rhEGF 처리 후 정상 섬유모세포에서는 EGFR의 Tyr845 부위에서 인산화가 일어났고, AMC-HN3와 A431 세포주에서는 Tyr845, Tyr992, Tyr1045, Tyr1068 부위에서 인산화가 일어났다. AMC-HN3와 A431 세포주에서 rhEGF 처리에 의한 p38 발현에는 변화가 없었다. rhEGF 처리는 AMC-HN3와 A431 세포주에서 방사선민감도를 증가시켰고, 방사선 조사 후 γH2AX 발현을 증가시켰다.

결론: rhEGF 처리는 EGFR 과발현 암세포주에서 세포 사멸을 유도하며, 주된 기전은 세포고사로 판단된다. 또한 rhEGF에 의한 세포 사멸은 PI3K/Akt/mTOR 신호전달체계의 억제와 연관이 있었다. rhEGF는 EGFR 과발현 암세포주에서 방사선민감도를 증가시키며, 그 기전은 DNA 손상 수복 억제로 판단된다.

주요어: EGF, EGFR 과발현 암세포주, 세포고사, PI3K−Akt−mTOR 신호전달체계, 방사선민감도 증가

학번: 2004−30600