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Epidermal growth factor as a potential radiosensitizer in mouse xenograft model

마우스모델을 이용한 상피세포성장인자의 방사선 민감제로서의 기능 평가

2014년 2월

서울대학교 대학원

임상의과학과 방사선종양학 전공

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이 논문을 의학석사 학위논문으로 제출함

2013년 10월

서울대학교 대학원

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2013년 12월

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Epidermal growth factor as a potential radiosensitizer in mouse xenograft model

by

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A thesis submitted to the Department of Clinical Medical Sciences, Graduate School in partial fulfillment of the requirements for the Master of Science in Clinical Medical Sciences at Seoul National University College of Medicine

December 2013

Approved by Thesis Committee:

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ABSTRACT

Introduction: The conventional principle of epidermal growth factor (EGF) – epidermal growth factor receptor (EGFR) interaction involves increased cell growth, proliferation and differentiation. However, previous experimental studies have shown the anti-tumor effect and the possibility of radiosensitizing effect of exogenous EGF. This study aimed to evaluate if EGF could enhance the radiation sensitivity *in vivo*.

Methods: Balb/c-nu mice with A431 human epidermoid carcinoma were divided into five groups: group I (no treatment), group II (EGF for 6 days), group III (EGF for 20 days), group IV (radiotherapy (RT)), and group V (RT with concomitant EGF for 6 days) (n=8 for each group). EGF was given by intraperitoneal injection (5mg/ kg) once daily, and the total RT dose was 30 Gy with a fraction size of 5 Gy/ fx at 24-h intervals. For the group of RT+EGF, EGF was injected concomitantly with RT. Each treatment was started when the tumors reached a volume of about 200mm^3, and the tumor volumes were measured every other day until day 23. The relative tumor volume (RTV) was calculated by dividing the tumor volumes at any time by the tumor volume at the start of treatment. Mice of group I-V were sacrificed on day 0, 12, 23 to obtain tumor and major organ tissues. Histologic examinations of tumor, liver, lung, and kidney tissues were performed from H&E staining, and immunohistochemistry (IHC) staining of caspase-3 was performed to assess EGF-induced apoptosis in tumor tissues.

Results: Mice with EGF for 6 daysshowed decreased tumor growth in comparison

with the control group. On day 13, the mean values of RTV for the group I and II were 9.06 and 7.23, respectively. However, the relative tumor volume approached to the level of the control group on day 23, and the differences over the duration of follow-up were not statistically significant (P = 0.550). In the group IV and V, differences in tumor volume were observed after the emergence of the 1st minimal value of tumor volume (0.92 ± 0.05 on day 9 for group IV; 0.69 ± 0.11 on day 13 for group V). Both groups showed subsequent increases in tumor volumes, however, the slope of the tumor re-growth phase was higher in group IV than group V. The differences were statistically significant (P = 0.034). In contrast to the group II,decrease in tumor volume was maintained until the end of follow-upin mice with EGF for 20 days. On day 23, the mean values of relative tumor volume of group I, II, and III were 17.63, 16.48, and 10.64, respectively (P< 0.001). In IHC of cleaved caspase-3 antibody, EGF-treated mice of group II and III showed stronger staining results than the control group. Also in the group IV and V, the intensity of the immunostaining was higher in group V. There were no abnormal histologic findings in H&E slides of major organs, such as liver, lung, and kidney.

Conclusions: The EGF-induced effect of tumor growth suppression was observed in A431 human epidermoid carcinoma of mouse xenograft model. Concomitant use of EGF induced tumor growth delay more, and it showed the potential as a radiosensitizer in the design of fractionated RT.

Keywords: epidermal growth factor, radiosensitizer, anticancer agent, *in vivo*, apoptosis

Student Number: 2012-22723

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INTRODUCTION

Epidermal growth factor (EGF) is a single polypeptide of 53 amino acid residues, and it is involved in cell growth, proliferation and differentiation (1). It stimulates target cells by binding to epidermal growth factor receptor (EGFR) which is a transmembrane receptor with an intrinsic tyrosine kinase activity. The interaction of EGF-EGFR induces dimerization and autophosphorylation of the receptor, and it regulates the expression level of a variety of transcription factors through multiple signaling pathways, such as RAS/ERK, PI3K/AKT, MAPK, and JAK/STAT (2).

Contrary to the conventional concept of action of EGF, treatment of EGF is sometimes associated with increased cell death. Kwon et al. studied the effect of EGF in a dose-dependent manner, and they found out that EGF inhibited proliferation of a cancer cell line with the high EGFR expression, not in normal fibroblast (3). Cao et al. demonstrated that nM level of EGF decreased cell adhesion and induced apoptosis in A431 cell line, and it was recognized that EGF could induce apoptosis which was related to cell cycle arrest at G1 check point with alterations in expression levels of regulatory proteins (4). Also, the binding of EGF leads to the internalization of EGFR, and this structural modification was known to be a specified process of EGF-induced apoptosis (5, 6). More recently, the endocytoplasmic regulation or trafficking of EGF-EGFR complex and the potential associations with STAT1, Src, and p38 MAPK were being discussed (7-10).

Targeting agents of EGFR-related signaling pathway have been novel therapeutic agents and increasingly used in lung, head and neck, pancreatic, and colorectal

cancers (11). It was demonstrated that the inhibition of EGFR signaling pathways leads to cytostatic and cytotoxic effect and decreased repopulation of tumor cells (12). In addition, the combined use of the EGFR inhibitors and radiotherapy has been studied, and it has been expected that EGFR inhibitors can enhance the therapeutic efficacy of fractionated irradiation from preclinical and clinical studies (13-15). Based on these principles, I focused on the contradictory concept, EGF-induced anti-tumor effect. Although earlier studies showed that the presence of EGF after or prior to irradiation enhanced the radiosensitivity of human squamous cell carcinoma cell lines *in vitro*, there have been few studies investigating the *in vivo* effect (16-18).

Therefore, this study aimed to evaluate the tumor growth suppression and radiation-sensitizing effect of EGF in mouse xenograft model with human cancer cell line. In addition, potential adverse systemic effects of EGF on major normal organs (liver, lung, and kidney) in the mouse xenograft model were examined.

MATERIALSAND METHODS

1. Cell line

A431 human cancer cell line was used, which is originated from human vulvar epidermoid carcinoma with high expression of EGFR. Cell line was purchased from American Type Culture Collection (ATCC). The cells were maintained in DMEM (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), and grown in an incubator with humidified atmosphere of 95% air/ 5% CO_2 at 37.5℃.

2. Study reagent

Recombinant human EGF was provided by Daewoong Pharmaceutical Company (Seoul, Korea) in the form of powder. The powder was diluted in PBS with the concentration of 1 mg/ml and stored at -70℃.

3. Animals

This experiment was approved by the Institutional Animal Care and Use Committee (IACUC), and performed at the Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea (an AAALAC accredited facility). National Research Council (NRC) guidelines for the care and use of laboratory animals were observed. Male 5-week old Balb/c-nu mice were used in this study. The animals were maintained in the facility in Biomedical Research Institute of Seoul National University Hospital, which was approved by the Korean Food and Drug

Administration and complies with the regulations and standards of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University. The mice housed under pathogen-free conditions with controlled humidity (40% - 60%) and temperature (20 - 24℃). A light program regulated a 12/12-h light dark-rhythm, with lights on from 8 a.m. to 8 p.m. The mice were kept in individual ventilated cage (IVC) system on sawdust bedding. Standard mouse diet and filtered city tap water from standard Perspex drinking bottles were provided *ad libitum*.

4. *In vivo* tumor models

$5x10^5$ A431 cells were injected subcutaneously into the right hind leg of the mice. When the tumors reached a volume of 200 mm^3 at about 7-9 days after the inoculation, mice were divided into 5 groups (n=8 for each group). Also, additional two mice (n = 2) in each experiment without inoculation of tumor cells received intraperitoneal EGF for 20 days. They were monitored for 6 months without other interventions to evaluate potential adverse effect of EGF on major organs. The control group did not receive any treatment, and others received the treatment of EGF (for 6 days or 20 days), irradiation (RT), or RT plus concomitant EGF (for 6 days) (Figure 1). The EGF was given by intraperitoneal injection (5 mg/kg) once daily. RT was delivered to the tumor once daily using 6-MeV photon energy (Clinac 6/100, Varian, PaloAlto, CA, USA) at a dose rate of 270 MU/min. The fraction size was 5 Gy/ fx and the total RT dose was 30 Gy. For the immobilization of the body

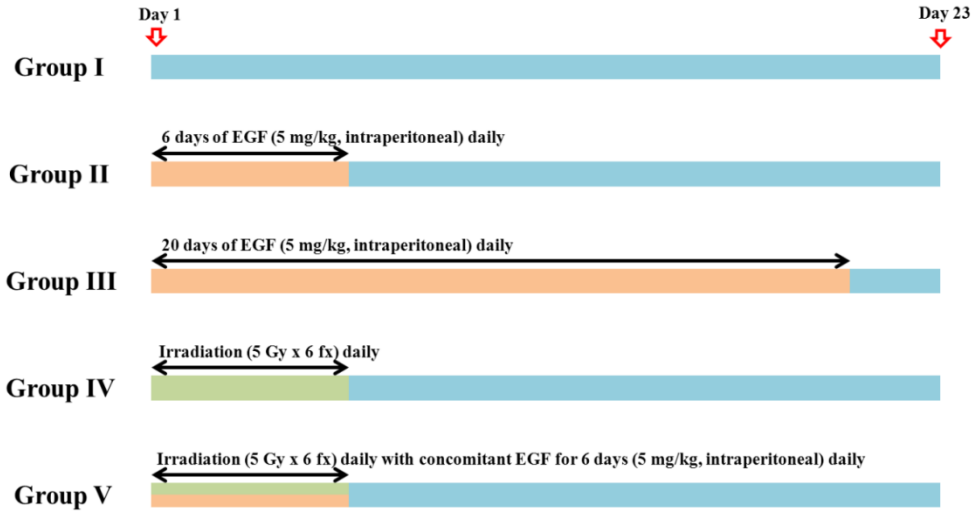

Figure 1. Treatment groups, dose and schedules in A431 xenograft models of nude mice

and the leg tumors, a custom-made acrylic device was used (Figure 2). For the group of RT + EGF, EGF was injected concomitantly with the irradiation with the same dose. The day 1 was defined as the start date of each treatment. The tumor volumes were measured until the day 23.

5. Measurement of tumor volume

Tumor size was measured every other day using a vernier caliper by two independent researchers (Lim and Jeon) until day 23. Tumor volume was calculated according to the formula of 1/2 x length x width^2 (mm^3). Mice of group I-V were sacrificed on day 0, 12, 23 to obtain paraffin blocks of tumor tissues and major organs, such as liver, lung, and kidney. The experiments were independently repeated three times. The data were expressed as the absolute tumor volume ± standard deviation (SD).

6. Histologic examination

Tumor and major organ tissues of group I-V were examined in H&E staining slides. The morphologic findings of liver, lung, and kidney were compared to assess the systemic impact of exogenous EGF. In addition, immunohistochemistry staining of EGFR was performed to confirm the expression level of EGFR in A431 cells. Immunohistochemistry of cleaved caspase-3 was also used for analysis of EGF-induced apoptosis in tumor tissues. A single pathologist (Koh) reviewed the immunohistochemistry results without prior knowledge of treatment outcome.

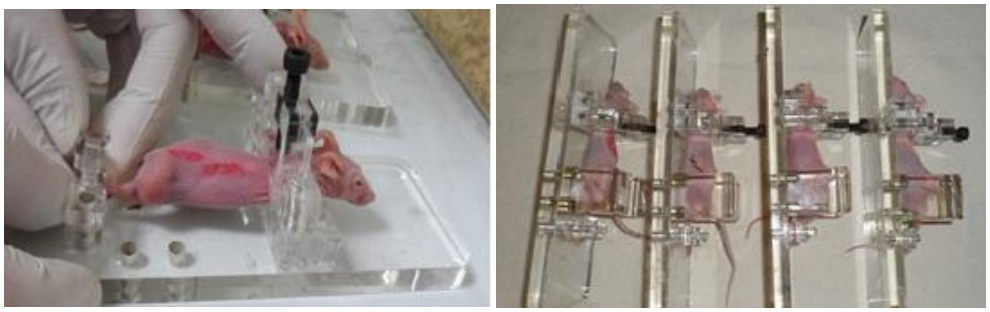

Figure 2. Immobilization with custom-made acryl device for RT

Digital photomicrographs were obtained at x200 and x400 magnification. The intensity of expression was reported in a semi-quantitative manner.

7. H&E and immunohistochemistry staining of FFPE sections

Five-micrometer-thick paraffin-embedded tumor sections were cut and deparaffinized in DAKO PT Link, and stained with H&E or labeled with primary antibodies, anti-EGFR (#4267, Cell Signaling, 1:50) and anti-cleaved caspase-3 (#9661, Cell Signaling, 1:50) with antigen retrieval process which was performed at 97℃. After endogenous blocking was done with 3% H_2O_2, the secondary antibodies of HRP labeled polymer anti-rabbit were applied. The samples were developed with DAKO REAL^{TM} DAB+ chromogen and treated with Mayer's Hematoxylin.

8. Statistical analysis

The tumor volume data were presented as mean ± standard deviation (SD) of absolute tumor volume (mm^3) or relative tumor volume which was defined as the value of ratio between the final volume and the initial volume from three independent experiments. The relative differences in relative tumor volume were analyzed by linear mixed model analysis. The *P*-value < 0.05 was considered to be statistically significant. The mean data of three independent experiments were included in the analysis.

RESULTS

The anti-tumor and radiosensitizing effect of EGF

Table 1 shows the results of absolute tumor volume of all experimental groups. To compare the therapeutic outcome of the experimental groups, relative tumor volumes were represented in tumor growth curves (Figure 3 and 4). First, the mice which were treated with EGF for 6 days showed decreased tumor growth in comparison with the control group (Figure 3). The definite differences were observed since the day 13. At that time, the mean values of relative tumor volume for the group I and II were 9.06 and 7.23, respectively. However, the slope of the curve of group II started to increase. The relative tumor volume approached to the level of the control group on day 23, and the mean values of relative tumor volume of group I and II were 17.63 and 16.48, respectively. The differences in tumor volume over the duration of follow-up were not statistically significant ($P = 0.550$).

In the group of RT and RT withconcomitant EGF for 6 days, differences in relative tumor volume were observed after the emergence of the 1st minimal value in the mean relative tumor volume (Figure 3). Whereas the group IV showed the minimal mean value on day 9 (0.92 ± 0.05), the tumor volume of group V continuously decreased until day 13 with the minimal mean value (0.69 ± 0.11) which was lower than the group IV. After the minimum points, relative tumor volumes started to increase subsequently both in the group IV and V. The slope of the tumor re-growth phase was higher in group IV than V until the end of follow-up. The differences were statistically significant ($P = 0.034$).

Table 1. Mean absolute tumor volume of the five groupsduring treatment and follow-up

day	Absolute tumor volume (mean ± SD*) (mm^3)				
	I (control)	II (EGF x 6)	III (EGF x 20)	IV (RT only)	V (RT +EGF)
1	216.65±24.87	195.45±13.45	210.25±26.56	201.22±24.73	218.06±12.62
3	385.42±68.11	295.94±28.17	318.22±28.86	338.85±53.35	385.24±19.92
5	555.96±48.03	512.28±83.51	563.90±29.89	323.26±57.04	344.08±23.85
7	769.30±96.61	673.68±36.14	740.70±76.61	248.58±48.28	270.19±20.39
9	1094.04±137.00	964.54±112.07	999.15±204.72	185.32±26.47	222.62±19.64
11	1375.10±71.79	1206.19±134.20	1131.37±101.11	240.52±20.27	191.93±22.20
13	1953.74±136.96	1414.52±156.74	1264.57±52.78	296.47±50.75	150.64±26.52
15	2415.31±179.67	1772.71±234.71	1468.60±180.76	362.11±58.35	185.46±63.77
17	2723.29±251.43	2048.02±276.69	1553.24±304.21	484.48±108.51	240.04±74.12
19	3127.48±295.22	2468.86±225.79	1744.07±347.33	601.90±123.49	358.78±77.07
21	3440.04±364.94	2884.01±139.29	1971.36±398.34	731.05±150.65	412.34±89.71
23	3820.99±497.50	3218.63±209.65	2248.96±432.63	903.18±149.06	484.19±82.59

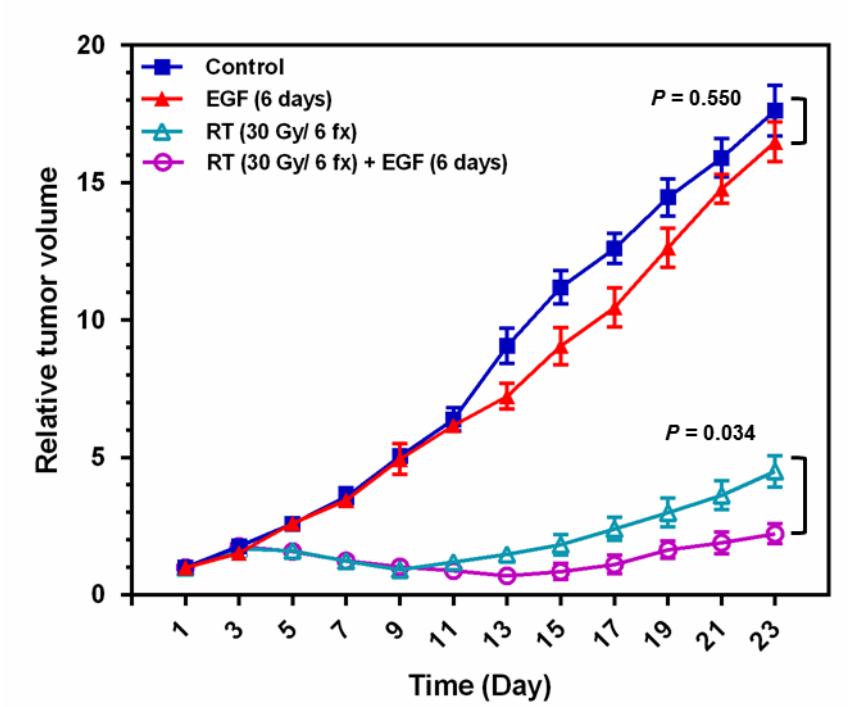

Figure 3. Changes of mean values of relative tumor volume in the group I (control) vs. II (EGF for 6 days) and IV (RT (30 Gy/ 6 fx)) vs. V (RT (30 Gy/ 6 fx) with concomitant EGF for 6 days) after treatment

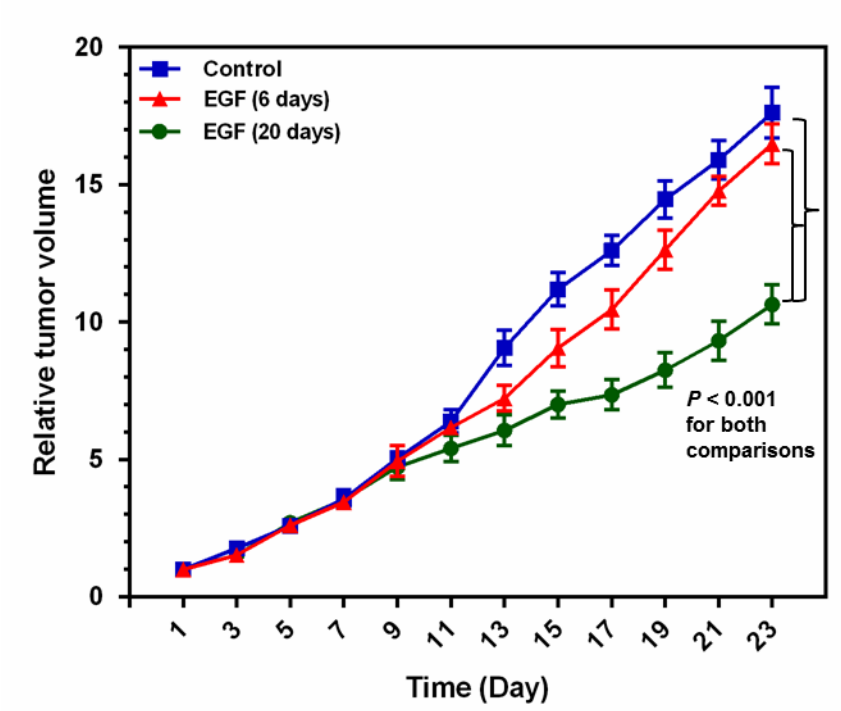

Figure 4. Changes of mean values of relative tumor volume in the group I (control), II (EGF for 6 days), and III (EGF for 20 days) after treatment

Figure 4 shows differences in relative tumor volume among the group I, II, and III. In contrast to the group of EGF for 6 days, the anti-tumor effect of the group of EGF for 20 days was maintained until the end of follow-up. On day 23, the mean values of relative tumor volume of group I, II and III were 17.63, 16.48, and 10.64, respectively.The decreased tumor volume of group III compared to group I and II were statistically significant ($P < 0.001$ for both comparisons).

EGFR expression of A431 cells

Immunohistochemistry of EGFR displayed strong membrane staining in A431 tumor cells (Figure 5). The intensity and pattern of EGFR expression were not different among the group I-V. The EGFR scoring system used in this study is listed in Table 2 (19).

EGF-induced apoptosis

In the results of immunohistochemistry of cleaved caspase-3 antibody, EGF-induced apoptosis was found out in tumor tissues. The staining intensity of each group is listed in Table 3. The apoptotic activity of cleaved caspase-3 was defined as the proportions of apoptotic cells in the resected tumor tissue on each slide. In group I, II, and III, cytoplasmic uptake of the antibody was stronger in EGF-treated mice than the control group. In comparison with group IV and V, the intensity of immunostaining was also higher in group V (Figure 6).

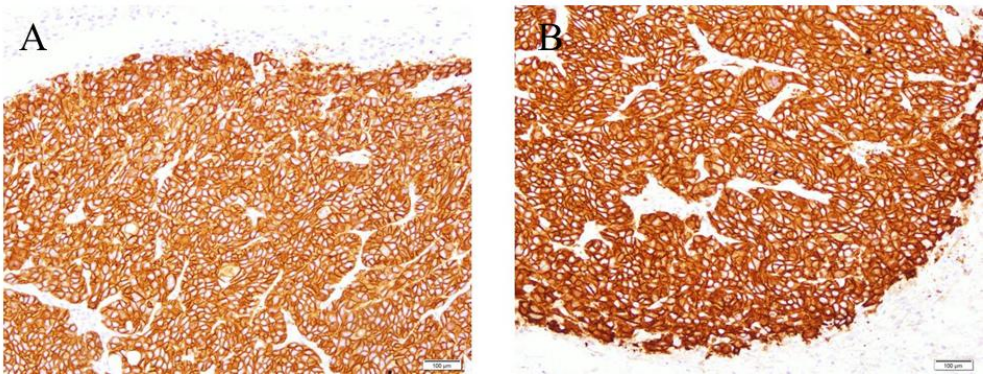

Figure 5. Immunohistochemistry of anti-EGFR antibody: paraffin-embedded tumor sections of group I (control) (A) and III (EGF for 20 days) (B). A431 (human epidermoid carcinoma) tumor tissues from all of the five groups showed high expression level of EGFR (x 200, Day 0)

Table 2. Scoring system of EGFR immunohistochemistry

Quantitative evaluation		Staining intensity		Final evaluation	
Immunopositive cells (%)	Score	Intensity	Score	Combined score	Expression level
<1	0	Weak	1	0-2	Low
1-20	1	Moderate	2	3-5	Intermediate
20-50	2	Strong	3	6-7	High
50-80	3	-	-	-	-
>80	4	-	-	-	-

Table 3. Immunohistochemistry of cleaved caspase-3 and EGFR of tumor tissues (Day 12)

	I (control)	II (EGF x 6)	III (EGFx20)	IV (RT)	V (RT+EGF)
EGFR	High	High	High	High	High
Cleaved caspase-3					
Apoptosis (%)	3	7	6	10	25

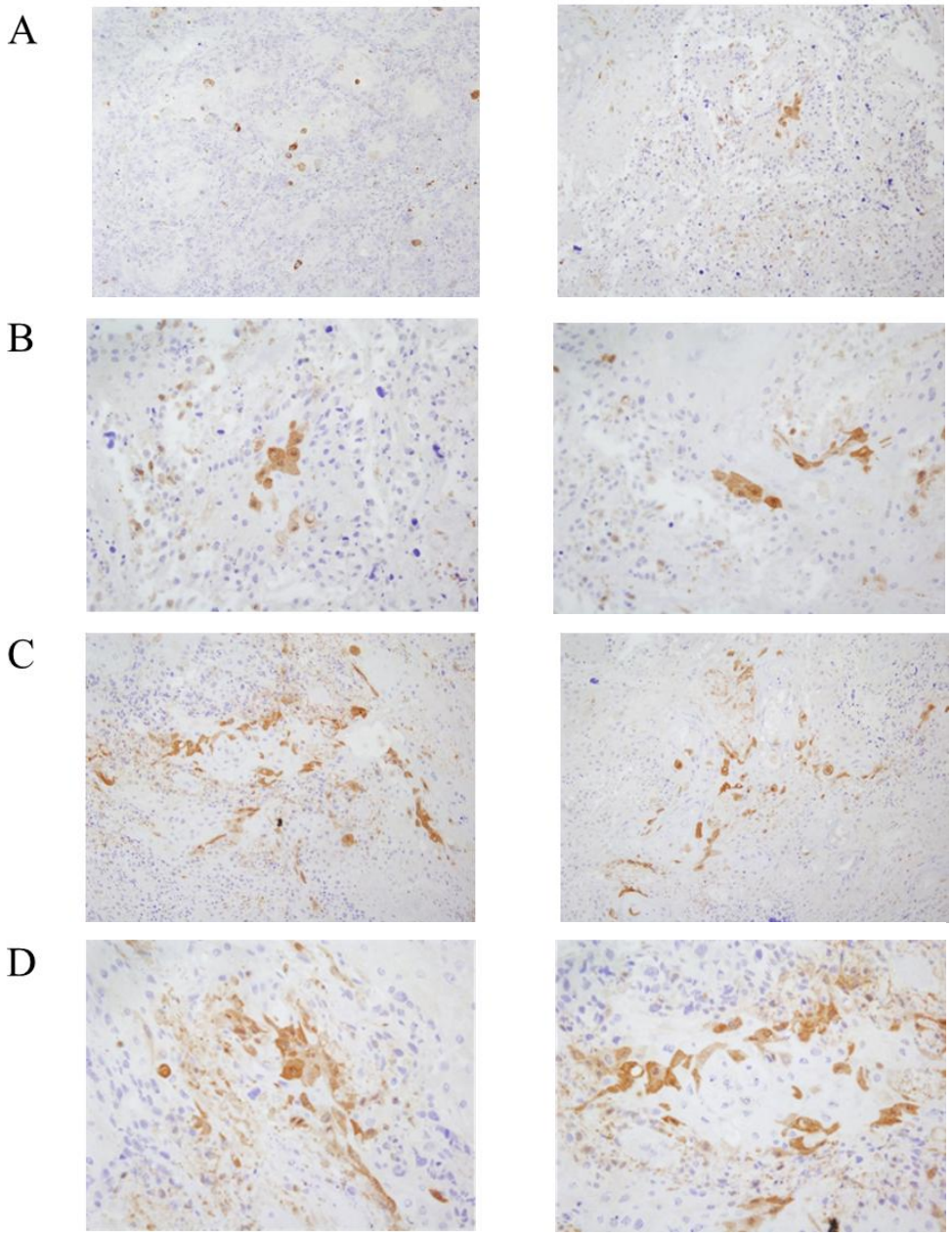

Figure 6. Immunohistochemistry of anti-cleaved caspase-3 antibody (paraffin-embedded tissue sections). Representative staining results of group IV (RT (30 Gy/ 6 fx)) (Day 12) (A: x200, B: x400) and group V (RT (30 Gy/ 6 fx) with concomitant EGF for 6 days) (Day 12) (C: x200, D: x400)

Impact of EGF on major normal organs

In the H&E slides of liver, lung, and kidney of mice sacrificed, there were no abnormal histologic findings in group II, III and V which were treated with EGF. Liver sections showed normal hepatic lobules and portal areas with intact perilobular and intralobular systems. Also, in lung sections, normal bronchiolar and alveolar structures were observed, and I could not find any abnormality in glomerulus, capillary and renal ductal structures in kidney sections. Although there wasone death event from each experiment at the end of follow-up (Day 22-23), abnormal histologic findings were not observed in liver, lung, and kidney tissues (Figure 7). Also, there were no detrimental events in group V (RT+EGF). Considering the additional mice treated with EGF for 20 days in the absence of tumor inoculation, there were no death events, and abnormal histologic features in major normal organs were not observed (Figure 8).

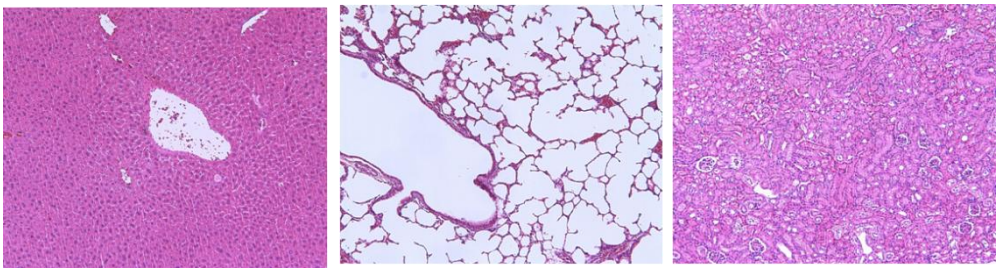

Figure 7. H&E staining of major normal organs (liver, lung, and kidney, respectively) of a mouse with sudden death(x100).

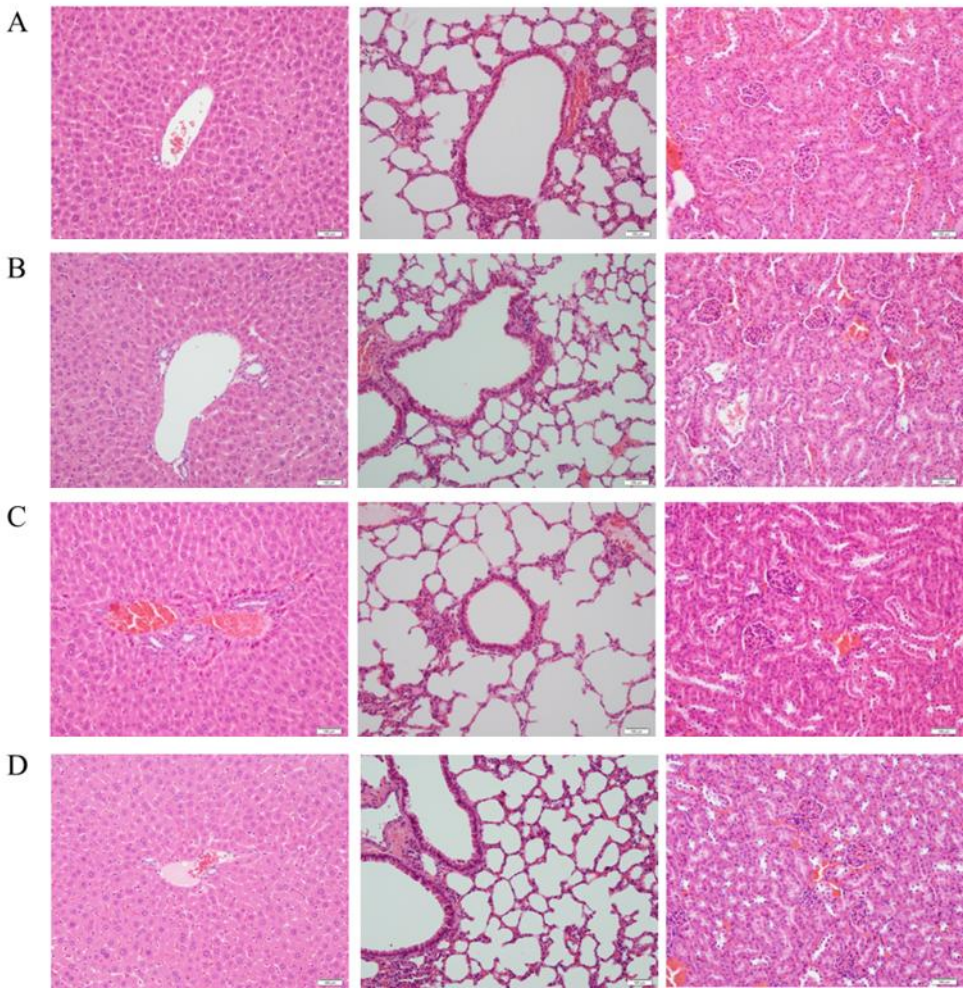

Figure 8. H&E staining of major normal organs. Microscopic findings of liver, lung, and kidney, respectively (x200): Group I (control) (Day 23) (A), Group III (EGF for 20 days) (Day 23) (B), Group V (RT (30 Gy/ 6 fx) with concomitant EGF for 6 days) (Day 23) (C), and additional mice (EGF for 20 days without tumor inoculation – after 6 months follow-up) (D)

DISCUSSION

The anti-tumor effect of exogenous EGF has been demonstrated for several decades. However, most previous results were based on *in vitro* experiments. This study aimed to establish the EGF-induced cell death in mouse xenograft models and its potential as a radiation sensitizer in the design of fractionated irradiation.

In comparison with control group, the slope of tumor growth curve was lower in group II and III, and the final tumor volume of control group was significantly higher at the end of follow-up. However, the tumor size of group II and III also continued to grow, and an absolute decrease in tumor volume was not observed over the entire follow-up duration. That is, the injection of exogenous EGF induced tumor growth suppression, but this was not clearly associated with tumor shrinkage.

Choi J et al. also evaluated the anti-tumor effect of EGF in mouse xenograft models (20). The study compared potential cytotoxic effects of EGF (i.p., 1 mg/kg, every other day, three times) and cisplatin (i.v., 5 mg/kg, once) using subcutaneously inoculated human cancer cell lines including A431 cells. Although the study design was different from the present study, similar patterns of growth suppression by EGF were observed. From the data of relative tumor volume, not only the cancer cells with high expression level of EGFR, but cell lines with lower EGFR expression also showed tumor suppression with the intraperitoneal EGF. The authors concluded that EGF was effective in the reduction of tumor burden and may be a cytotoxic agent to certain kinds of tumors.

Although the anti-tumor effect of EGF for 6 days was not statistically significant

compared to control group, the group of RT with concomitant EGF (30 Gy/ 6 fx, for 6 days) showed significant difference compared to RT alone group in the tumor growth curve. That is, intraperitoneal injection of EGF for 6 days was not sufficient to obtain anti-tumor effect at the end of follow-up, however, EGF for 6 days with fractionated RT resulted in prolonged decrease in tumor volume and less tumor re-growth until day 23. Therefore, the concomitant use of EGF with RT enhanced the radioresponse of tumor and its anti-tumor effect decreased tumor repopulation after completion of RT.

The current findings of this study are coincided with the previous *in vitro* results.Kwok and Sutherland initially reported that exogenous EGF after or continuously before, during, and after irradiation enhanced the radiosensitivity of CaSki cell line, the human squamous cell carcinoma *in vitro* (16). From radiation dose-response curves, the authors demonstrated that EGF enhanced radiosensitivity*in vitro* with reduction in the shoulder region of the cell survival curves, and the maximum effect appeared with 10 ng/mL of EGF. They also extended the results into other cell lines, such as A431 and HN5, and found out that the EGF-related enhancement of irradiation was higher in G1 phase which showed wider shoulder region than other phases (17, 21). However, few studies have reported the impact of EGF on the radiosensitivity so far.

More recently, Kwon EK et al. designed *in vitro* and *in vivo* studies to evaluate the effect of EGF on cell proliferation and radiation survival (3). From the results of clonogenic assays, EGF suppressed tumor growth in the cancer cell line with high expression of EGFR, and the inhibitory effect was more evident in higher

concentrations of EGF (1nM or more). When EGF was combined with irradiation *in vitro*, EGF enhanced the cell killing effect of irradiation in cancer cell lines, but not in the normal fibroblast cells. Also, in the study of mouse models with EMT-6 (mouse mammary sarcoma), the treatment of recombinant human EGF (1 mg/ kg for 7 consecutive days, three times a day) was combined with irradiation (10 Gy or 20 Gy of single dose). While there were no significant differences in tumor growth rates among the experimental groups, the relative tumor volume of the group of EGF alone was maintained to be lower than that of the control group.However, the authors concluded that further studies might be needed to investigate such effect in human cancer cell lines, not in the mouse tumor.

It was also noticeable that the overall growth inhibition was more evident in the group III than group II. Although the tumor volume of the EGF for 6 days eventually approached to the level of the control group, the anti-tumor effect of EGF for 20 days was maintained. These results suggest that longer duration of dailyEGF injections induced more tumor suppression in group III. Therefore, an adequate exposure to EGF may be needed to obtain an observable level of EGF-induced tumor suppression *in vivo*. Since the differential anti-tumor effect due to total duration of EGF treatment has not been evaluated in other studies, further studies are needed.

Caspase-3 is one of the key molecules of apoptosis pathway. This study suggested the histologic evidence of increased apoptotic cells in mice treated with EGF, which was correlated with the results of increased radioresponse and smaller tumor volumes. Song JY et al. performed *in vitro* study with A431 cells and demonstrated

that sustained activation of EGFR by EGF was related to activation of caspase-3 together with a time-dependent cleavage of caspase-8 from protease assay and western blot analysis (5). They concluded that EGF-mediated apoptosis can be induced by both mitochondrial and non-mitochondrial pathways. Also, Chiu B et al. performed *in vitro* study with neuroblastoma cell lines and they demonstrated that maximal level of total caspase-3 expression was obtained in the condition of incubation with EGF (5 ng/ml) (22). In this study, it is noticeable that the increased uptake of cleaved caspase-3 in the groups treated with EGF was observed in mouse xenograft models *in vivo*.

The impact of EGF on major normal organs was also evaluated, and the intraperitoneal injection of EGF did not induce adverse histologic changes in liver, lung, and kidney. While death events existed on day 22-23 in the group I-II, there were no events in the combination of RT + EGF.In addition, theextra mice treated with EGF for 20 days without tumor inoculation survived for 6 months without abnormal histologic findings. Thus, the death events seemed to be associated more with increased tumor burden, not the intraperitoneal EGF treatment.

Despite the results of anti-tumor and radiosensitizing effect of EGF, its possibility to be a practical treatment modality is still uncertain. This study did not investigate the potential systemic effect of EGF on other important organs, such as small bowel, large intestine, and brain. Since there have been few studies to evaluate toxicities of exogenous EGF *in vivo*, other adverse impact of EGF cannot be excluded. Therefore, to predict therapeutic utility of EGF treatment, more studies are needed to evaluate the exact toxicity profiles.

In conclusion, the anti-tumor effect of exogenous EGF in mouse xenograft models of A431 cell line is confirmed. Also, concomitant use of EGF induced tumor growth delay more compared to RT alone, and the potential role of EGF as a radiation sensitizer could be suggested. Beyond the original knowledge of increased tumor cell proliferation and pro-survival effect of EGF-EGFR interaction, a new concept of EGF-induced tumor suppression is noticeable. As far as I know, this is the first study which investigated the combined use of EGF and RT and its radiosensitizing effect in the design of fractionated irradiation. Further studies are needed for more information of optimal concentration of EGF and its potential systemic toxicities *in vivo*.

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국문 초록

목적: 상피세포성장인자는 상피세포성장인자 수용체와의 상호작용을 통해 세포 성장, 분열, 분화 등을 증가시키는 것으로 잘 알려져 있다. 하지만 이전 시험관내 연구 결과들을 통해 높은 농도의 상피세포성장인자가 종양 억제 효과는 물론 방사선 감수성을 증가시킬 수 있음이 보고된 바 있다. 이번 연구는 생체내에서 상피세포성장인자가 종양의 방사선 민감도를 증가시킬 수 있는지에 대해 알아보고자 하였다.

방법: Balb/c-nu 마우스에 A431세포주를 이식 후 총 다섯 가지의 군으로 나누었다: I (대조군), II (상피세포성장인자를 6일간 주입), III (상피세포성장인자를 20일간 주입), IV (방사선 치료), V (방사선 치료와 동시에 상피세포성장인자를 주입). 각 실험군 별로 8마리씩 배정했다. 상피세포성장인자는 5 mg/ kg의 농도로 하루에 한 번 복강 내 주입을, 방사선 치료는 총 조사 선량 30 Gy (5 Gy/ fx)으로 하루에 한 번 시행했다. 종양 평균 용적이 200 mm^3에 도달했을 때 각각의 치료를 시행했고, 종양의 용적은 2일에 한 번씩 23일이 지난 시점까지 측정했으며, 종양 조직과 주요 장기 (간, 폐, 신장) 조직을 적출하기 위해 0일, 12일, 23일에 각 실험군 별로 1마리씩 희생시켰다. 상피세포성장인자의 영향을 알아보고자 간, 폐, 신장 조직에서 H&E 염색 후 조직학적 소견을 관찰했고, 상피세포성장인자에 의한 종양의 세포자멸사를 평가하기 위해 caspase-3의

면역조직화학염색 결과를 관찰했다.

결과: 실험군 II는 I에 비해 종양 성장이 감소되었으며, 치료 후 13일이 지난 시점에서 각 실험군의 상대적 종양 용적은 9.06과 7.23 이었다. 하지만 23일 시점에 가까워지면서 종양 용적이 실험군 I의 수준으로 증가했으며, 추적 관찰 기간 동안의 실험군 I과 II 간 종양 용적 차이는 통계적으로 유의하지 않았다 (P = 0.550). 실험군 IV와 V의 경우, 방사선 치료 후 가장 낮은 상대적 종양 용적 수치를 보일 때 (IV: 9일 째 0.92 ± 0.05, V: 13일 째 0.69 ± 0.11)까지 종양 용적의 전체적인 변화는 두 군 간에 비슷한 양상을 보였다. 이후 두 실험군에서 모두 종양 용적의 재증가를 보였는데, 실험군 IV에서 V에 비해 더 가파른 증가 양상을 보였다. 이 두 군간 종양 용적의 차이는 통계적으로 유의했다 (P = 0.034). 실험군 II와는 달리, EGF로 20일간 치료한 경우에는 실험군 I과 비교했을 때의 종양 용적 감소가 마지막 추적 관찰 시점까지 유지되는 양상을 보였다. 23일 시점에서 실험군 I, II, III의 상대적 종양 용적은 17.63, 16.48, 10.64였으며, 추적 관찰 기간 동안 이들 간의 차이는 통계적으로 유의했다 ($P < 0.001$). Caspase-3의 면역조직화학염색 결과, 실험군 IV에 비해 V에서 면역 염색의 강도가 더 높았다. 모든 실험군에 있어서 간, 폐, 신장의 H&E 염색 상에 비정상적인 조직학적 소견은 관찰되지 않았다.

결론: A431 세포주를 이용한 마우스 모델을 통해 상피세포성장인자에 의

한 종양 성장 억제를 확인할 수 있었다. 방사선 치료와 상피세포성장인자를 함께 적용하는 것이 더 뚜렷한 종양 성장 지연을 가져왔고, 이를 통해 분할 방사선 치료에서 상피세포성장인자의 방사선 민감제로서의 역할을 기대할 수 있을 것이다.

주요어: 상피세포성장인자, 방사선 민감제, 항암제, 생체내, 세포자멸사
학번: 2012-22723