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Radiosensitizing effect of Lapatinib in HER2-positive breast cancer cells

HER2 양성 유방암 세포에서 Lapatinib 의 방사선 감작효과

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유 도 솔

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ABSTRACT

Introduction: Overexpression of human epidermal growth factor receptor 2 (HER2) is found in about 20% of breast cancer patients. Trastuzumab has been widely used for the treatment of HER2 overexpressing breast cancer, but the incidence of brain metastases has increased due to its inherent limitation of crossing the blood-brain barrier (BBB). Thus, lapatinib, which has a low molecular weight and can cross the BBB, in combination with brain radiotherapy, will be a promising therapeutic strategy in the treatment of brain metastasis from HER2-positive breast cancer.

Materials and Methods: To explore the impact of lapatinib on radiation response, we conducted an *in vitro* experiment using SKBR3 and BT474 breast carcinoma cells exhibiting HER2/neu amplification. Clonogenic assay and western blot was used to investigate the radiosensitivity of lapatinib. Antibody against γH2AX was used to detect DNA damage. Modes of cell death were assessed with V-FITC/propidium iodide double staining, β-galactosidase staining, and LysoTracker Green staining.

Results: Pretreatment of Lapatinib led down-regulation of p-HER2, p-EGFR, p-AKT, and p-ERK. Lapatinib increased the radiosensitivity of SKBR3 (SER 1.21 at surviving fraction of 0.5) and BT474 (SER 1.26 at surviving fraction of 0.5) breast carcinoma cells, respectively. Lapatinib hindered repair of DNA damage as suggested by the prolongation of radiation-induced γH2AX foci and down-regulation of p-DNAPKcs. Increased radiation-induced apoptosis and senescence was suggested to be the major mode of cell death induced by lapatinib combined with radiation. Lapatinib didn't increase radiation damage of normal human astrocytes.

Conclusions: These findings suggest lapatinib potentiate radiation-induced cell killing in HER2-overexpressing breast cancer cells and could be a useful strategy to increase efficacy of radiotherapy.

Keywords: Breast cancer, Human epidermal growth factor receptor 2, Radiotherapy, Lapatinib

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LIST OF ABBREVIATIONS

BBB: blood–brain barrier

CR: complete response

DAPI: 4′,6–Diamidino–2–phenylindole

DMEM: Dulbeco’s modified Eagle’s medium

EGFR: epidermal growth factor receptor

FBS: fetal bovine serum

FITC: fluorescein isothiocyanate

HER2: human epidermal growth factor receptor 2

NHA: normal human astrocyte

PI: propidium iodide

PR: partial response

RT: radiotherapy

SER: sensitizer enhancement ratio

WBRT, whole brain radiotherapy

INTRODUCTION

Overexpression of human epidermal growth factor receptor 2 (HER2) is found in about 20% of breast cancer patients and is known to have a particularly aggressive natural history (1). In the last decade, the introduction of trastuzumab, an anti-HER2 monoclonal antibody, has improved the survival of HER2-positive patients dramatically when used in the adjuvant setting (2, 3). The addition of trastuzumab to neoadjuvant chemotherapy increases pathologic complete response rate and event-free survival in locally advanced breast cancer (4, 5). Even in metastatic breast cancer patients, trastuzumab improves response rate and survival (6, 7). However, the incidence of brain metastasis is significantly increased in HER2-positive breast cancer after treatment with trastuzumab, possibly due to the fact that trastuzumab enhances systemic control and prolongs survival, and thus clinically discloses brain metastasis (8). In HER2-positive metastatic breast cancer, despite receiving trastuzumab-based therapy, approximately 30% of patients develop brain metastasis (9-11). Intracranial disease progression, rather than extracranial disease, is the

cause of death in about half of patients with brain metastasis (9, 12). Therefore, in HER2-positive breast cancer patients with brain metastasis, control of intracranial disease is an important issue in terms of survival.

Lapatinib ditosylate (GW572016/Tykerb®; GlaxoSmithKline, Research Triangle Park, NC) is a reversible dual inhibitor of the intracellular tyrosine kinase domain of HER1 and HER2. Lapatinib is expected to be used for breast cancer patients with brain metastasis because of its theoretical ability to cross the blood-brain barrier (BBB) resulting from its very low molecular weight (581 Da) (13). There has been attempts to demonstrate the effect of lapatinib for brain metastasis in patients with HER2-positive breast cancer, but the response rate of lapatinib alone to the brain lesions was only 3-6% (14-20).

We hypothesized that the combination of radiotherapy (RT) and lapatinib will be an effective strategy for HER2-positive breast cancer. Although there have been several *in vitro* studies using lapatinib alone or as a combination with other chemotherapeutic agents, no preceding *in vitro* studies are available on the

additive effect of lapatinib with RT (21, 22). Recent *in vivo* study showed that more effective tumor regression could be achieved if lapatinib is used in combination with radiation (23).

In this study, we investigated the radio-sensitizing effect of lapatinib using HER2-postivie breast cancer cell lines with clonogenic assay. Subsequently, we conducted *in vitro* experiments identifying the modes of cell death by lapatinib and radiation. We also checked whether the lapatinib has a harmful effect on normal human brain cells.

MATERIALS AND METHODS

1. Cell lines and cell culture

We used two HER2 amplified breast cancer cell lines (SKBR3 and BT474, American Type Culture Collection, Rockville, MD) (24). Cells were grown in 75 cm^2 plastic tissue culture flasks at 37° C in Dulbeco's modified Eagle's medium (DMEM, Welgene, Daegu, Korea) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) in 5% CO_2. Immortalized normal human astrocytes (NHAs) were derived from fetal brains.

2. Pharmacologic inhibitor

Lapatinib ditosylate (GW572016/Tykerb®; GlaxoSmithKline, Research Triangle Park, NC, USA) is a dual tyrosine kinase inhibitor of the HER1 (also known as epidermal growth factor receptor, EGFR) and HER2 domain (25). Lapatinib ditosylate was dissolved into concentrated stock solutions in DMSO, stored at −20° C, and diluted at the time of use in culture medium. Control cells were treated with medium containing an

equal concentration of the drug carrier, DMSO.

In a study by Taskar et al, lapatinib was administered (100 mg/kg, 200 μL per mouse) to mice via oral gavage. At 2 hours after oral administration, blood lapatinib concentration was 5.59 $\pm$0.56 μM and lapatinib concentration from metastatic brain tissue was 0.71$\pm$0.61 μM (21).

Morikawa, et al. conducted a prospective study in patients with brain metastases from breast cancer. The patients received 1250mg of oral lapatinib daily for 2−5 days. At the time of brain tumor resection, serum lapatinib concentration was 2.4−6.5mcM and lapatinib concentration from metastatic brain tissue was 1−63.6mcM. (26). However, tissue concentrations are not significant as reference points, due to the variability and unpredictability of the BBB in brain metastasis. Thus, with reference to previous data using serum lapatinib concentration, we decided on 5 μM of lapatinib as our reference point.

3. Clonogenic assays

Equal numbers of cells were plated across the different

treatment groups for each radiation dose. A specified number of cells was seeded into each well of six-well culture plates and treated with lapatinib two hours before irradiation. The cells were irradiated with 4-MV x-rays from a linear accelerator (Clinac 4/100, Varian Medical Systems, Palo Alto, CA, USA) at a dose rate of 2.46 Gy/min and were incubated for 14-21 days for colony formation. Colonies were fixed with methanol and stained with 0.5% crystal violet. The number of colonies containing at least 50 cells was determined, and the surviving fraction was calculated. Radiation survival data were fitted to a linear-quadratic model using Kaleidagraph version 3.51 (Synergy Software, Reading, PA, USA). Each point on the survival curves represents the mean surviving fraction from at least three dishes. The sensitizer enhancement ratio (SER) was calculated as the ratio of the isoeffective dose at a surviving fraction of 0.5 in the absence of lapatinib to that in the presence of lapatinib.

4. Western blot analysis

Cells were washed, scraped, and resuspended in lysis buffer

(iNtRON Biotechnology, Seoul, Korea). Proteins were solubilized by sonication, and equal amounts of protein were separated on SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA, USA). Membranes were blocked in PBS containing 0.1% Tween 20 and 5% powdered milk, and probed with primary antibody directed against p-HER2 (Tyr1221/1222), p-EGFR (Tyr1068), p-AKT (Ser473), p-ERK (Tyr202/204), p-DNA-PKs (Thr2609), Rad51, caspase3, and LC3 (Cell Signaling Technology, Inc.). Monoclonal anti-β-actin antibody was used at a 1:5,000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were washed and incubated with a secondary antibody consisting of peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin G (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at a dilution of 1:2,000 for one hour. Antibody binding was detected using an enhanced chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ, USA) using the appropriate secondary antibody supplied with the kit.

5. Immunocytochemistry

Cells were grown and treated on chamber slides. At specified times after treatment with lapatinib and/or radiation, cover-slips were rinsed, and cells were fixed in 4% paraformaldehyde and permeabilized in methanol for 20 minutes. Cells were subsequently washed and blocked in PBS containing 2% bovine serum albumin for one hour. A primary antibody against γ H2AX (Cell Signaling Technology, Inc.) was applied to the cells and incubated overnight. Secondary fluorescein isothiocyanate (FITC) anti-rabbit antibody (Molecular Probes, Eugene, OR, USA) was applied and incubated for two hours. A 4′ ,6-Diamidino-2-phenylindole (DAPI) nuclear counter stain was applied at 1 μg/mL for five minutes.

For analysis of autophagy, cells were washed and subsequently stained with 1 mM LysoTracker® Green (Molecular Probes) diluted in PBS for 10 minutes at 24 hours after irradiation. Apoptosis was evaluated using annexin V-FITC/propidium iodide (PI) double staining. Slides were examined on an Axio Scope.A1 Imager fluorescent microscope. Images were captured and acquired using AxioCam MRc5 and the acquisition

software AxioVision v.4.4 (Carl Zeiss, Gottingen, Germany). The number of formation of γH2AX foci was counted under 100x power field and the number of Punta in a cell was counted under 400x power field.

6. Senescence β-galactosidase staining

Cellular senescence was evaluated by detecting the activity of β-galactosidase. Tumor cells were seeded in 8-well chamber slides, treated with lapatinib and/or irradiation, and then stained using a Senescence β-Galactosidase Staining Kit (Cell Signaling Technology, Inc.) according to the manufacturer's instructions. Cells were examined using a light microscope. The number of cells stained by β-galactosidase was counted under 100x power field.

7. Modified Boyden chamber assay

Cell invasion was assessed using a Transwell System (Corning, Rochester, NY, USA) that allows cells to migrate through 8-mm pores in polycarbonate membranes. The membranes were

coated with a 10-mg/well gelatin solution in DMEM without FBS and dried. Inserts containing cells were placed into 24-well plates in starvation medium. Cells were trypsinized, washed, and resuspended (5×10^6 cells/mL) in starvation medium. An aliquot of 10^6 cells was added to the upper chamber. The lower chamber was filled with 500 mL of DMEM without FBS. After 24 hours, the surface of the upper membrane was swabbed with a cotton-tipped applicator to remove non-invasive cells. Inserts were fixed in methanol for 10 minutes and stained with 1 crystal violet for two hours. The invasion rate was quantitated microscopically by counting the number of moved cells.

8. Statistical analysis

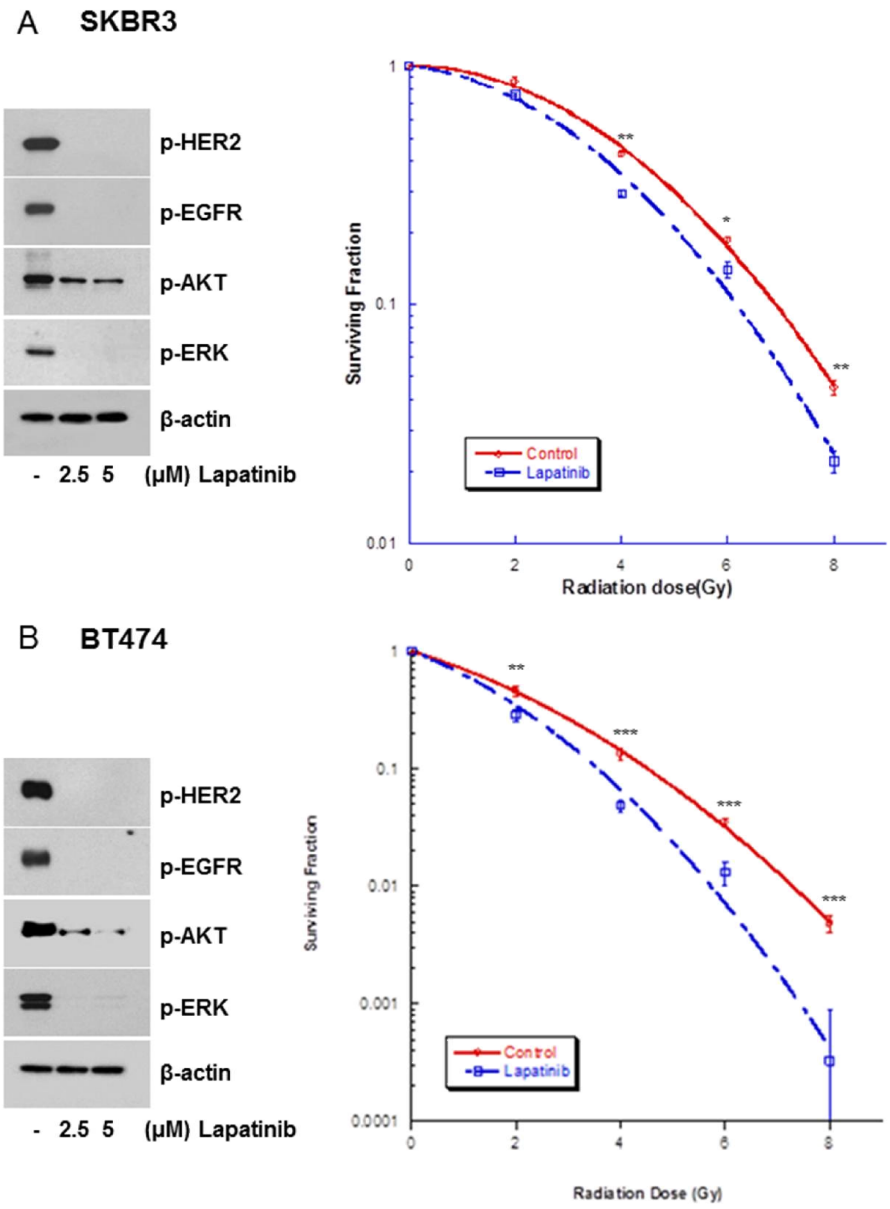
Data were analyzed for descriptive statistics using SPSS software (SPSS Inc, Chicago, IL, USA). The independent t-test was used to evaluate differences of each variable. A p value < 0.05 indicated statistical significance for all tests.

RESULTS

1. Lapatinib radio-sensitizes breast cancer cells overexpressing HER2.

To explore the impact of lapatinib on the radiation response, SKBR3 and BT474 breast carcinoma cells exhibiting HER2/neu amplification were treated with DMSO or lapatinib (5 μM). Lapatinib resulted in down-regulation of p-HER2, p-EGFR, and p-ERK (Figure 1, A and B). This was associated with increased radiosensitivity of SKBR3 and BT474 cells, as shown by decreased cell survival (Figure 1). The SER of lapatinib at a surviving fraction of 0.5 and 0.05 in SKBR3 cells was 1.21 and 1.11, respectively, and in BK474 cells was 1.258 and 1.279, respectively. Therefore, we concluded that lapatinib increases radiosensitivity of breast cancer cells overexpressing HER2.

Figure 1. Lapatinib radio-sensitized breast cancer cells overexpressing HER2.



(A) SKBR3 cells were treated with different concentration of lapatinib and the cell extracts were Western blotted with the indicated antibodies. Lapatinib attenuated the expression of p-

HER2, p-EGFR, p-AKT, and p-ERK in SKBR3 cells. The SKBR3 cells were treated with either DMSO or lapatinib (5 μM) and irradiated with 4-MV x-rays. The surviving fraction of cells treated with lapatinib was lower than that of control group.

(B) BT474 cells were treated with different concentration of lapatinib and the cell extracts were Western blotted with the indicated antibodies. Lapatinib attenuated the expression of p-HER2, p-EGFR, p-AKT, and p-ERK in BT474 cells. The BT474 cells were treated with either DMSO or lapatinib and irradiated with 4-MV x-rays. The surviving fraction of cells treated with lapatinib was lower than that of control group. $^{*}P \leq 0.05$. $^{**}P \leq 0.01$. $^{***}P \leq 0.001$.

2. Lapatinib hinders repair of DNA damage and may be involved in non-homologous end-joint repair.

Lapatinib caused marked increase of radiation-induced γH2AX foci indicating delayed repair of DNA damage compared to the control (Figure 2, A and B). To elucidate which repair mechanism is associated with the impact of lapatinib, whole cell extracts were Western blotted with p-DNAPK, Rad51, and β-actin. The expression of p-DNAPKcs, which is involved in non-homologous end-joining repair, was downregulated. Rad51, which indicates homologous recombination repair, was not affected by lapatinib. Thus, lapatinib hampers repair of DNA damage, which probably related to non-homologous end-joining repair.

(A)

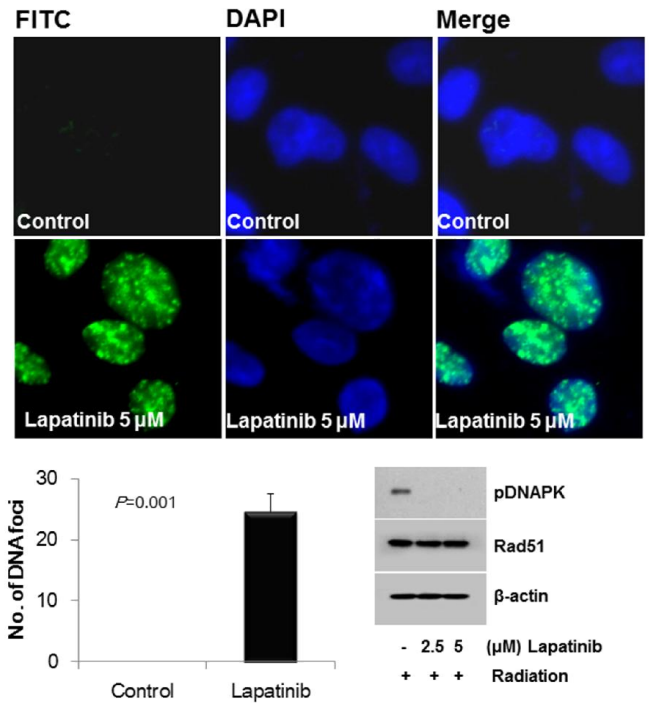
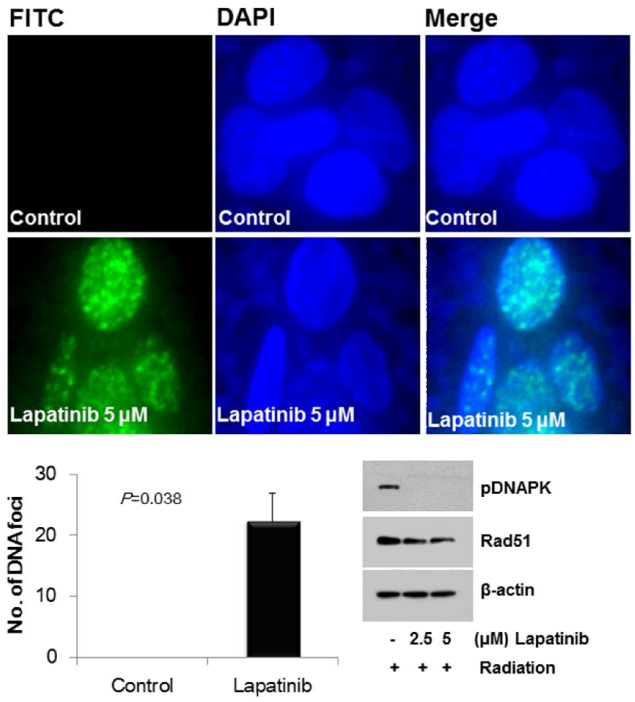


Figure 2. DNA damage repair: Lapatinib induced increase of γ H2AX foci.

(A) SKBR cells were treated with DMSO or Lapatinib (5 μM). The degree of DNA damage repair then determined by number of formation of γ H2AX foci 3 hours following 6 Gy of radiation. Lapatinib led increase of γ H2AX foci formation (*P*=0.001). The whole cell extracts were Western blotted with p-DNAPK, Rad51 and β-actin. The expression of p-DNA-PKcs was downregulated after add of lapatinib.

(B)



(B) BT474 cells were treated with DMSO or Lapatinib (5 μM). The degree of DNA damage repair then determined by number of formation of γH2AX foci 3 hours following 6 Gy of radiation. Lapatinib led increase of γH2AX foci formation (P=0.038). The whole cell extracts were Western blotted with p-DNAPK, Rad51 and β-actin. The expression of p-DNA-PKcs was downregulated after add of lapatinib.

3. Lapatinib potentiates radiation-induced apoptosis and senescence.

We next asked which mechanisms of cell killing would affect the cell's radiosensitivity to lapatinib. To this end, SKBR3 cells were treated with DMSO, lapatinib (5 μM), radiation (6 Gy), or lapatinib (5 μM) and radiation (6 Gy), and then stained with PI and antibodies against annexin V (Figure 3A). The number of apoptotic cells was then determined by double stained foci with annexin V and PI. Radiation alone and lapatinib alone did not appreciably increase apoptosis, but a significant increase in apoptosis was observed when they were used concurrently. Western blot using whole cell extracts also showed increased apoptosis when lapatinib was used in addition to radiation. Senescence was examined by detecting the activity of β-galactosidase and a notable change was observed in the cells treated with both radiation and lapatinib (Figure 3B). Thus, based on these assays, lapatinib induces both apoptosis and senescence of SKBR3 cells when used with radiation.

(A)

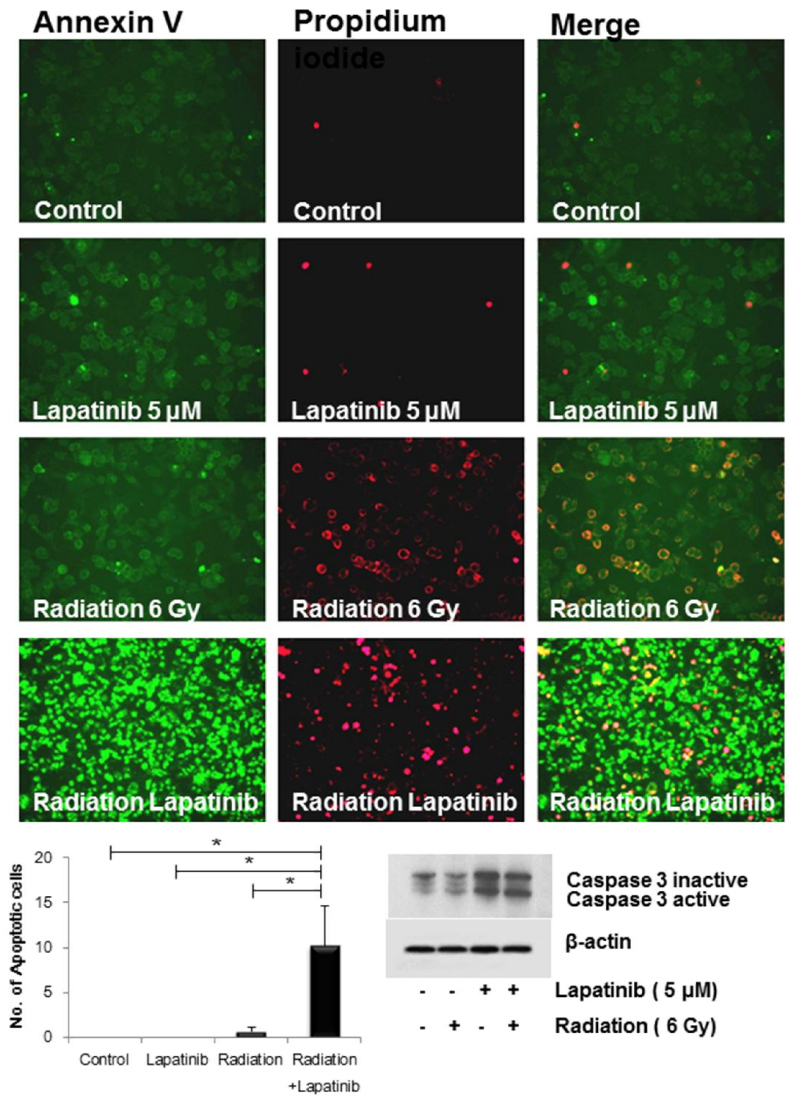
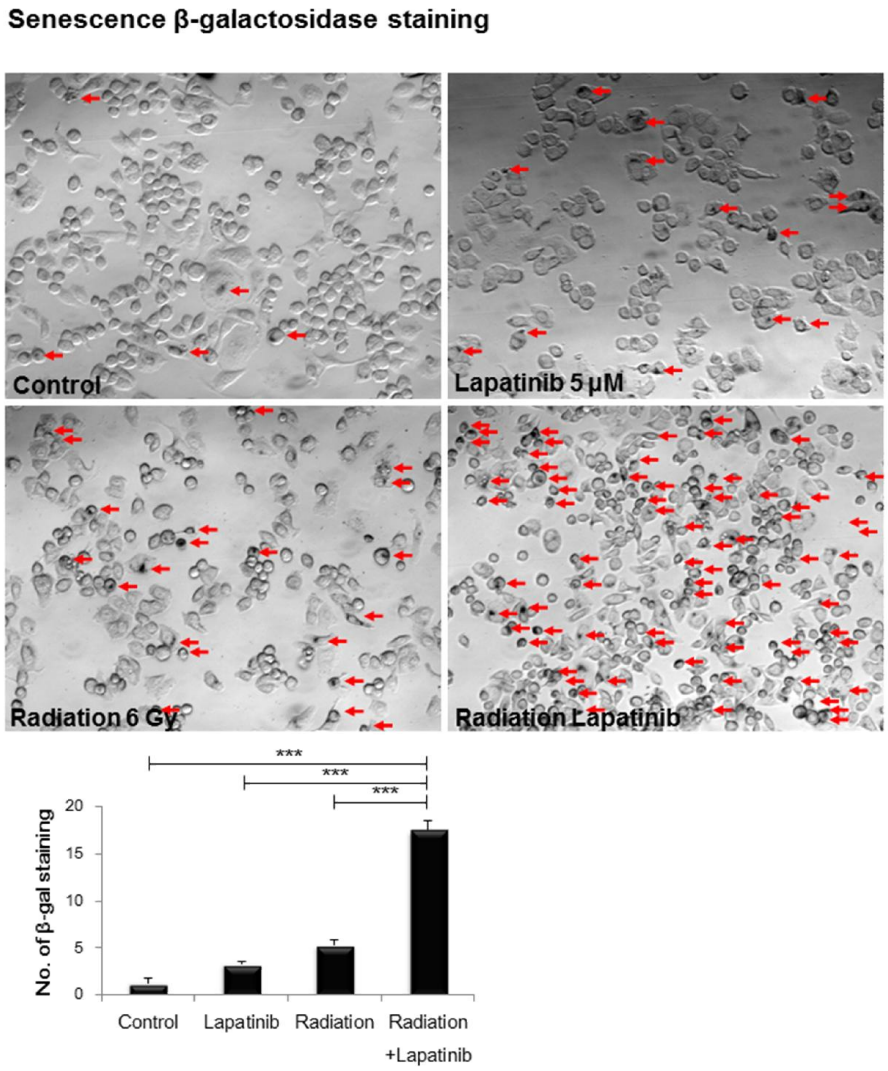


Figure 3. Lapatinib potentiated radiation-induced apoptosis and senescence.

(A) SKBR cells were treated with lapatinib (5 μM), radiation (6 Gy), or lapatinib (5 μM) and radiation (6 Gy), then stained

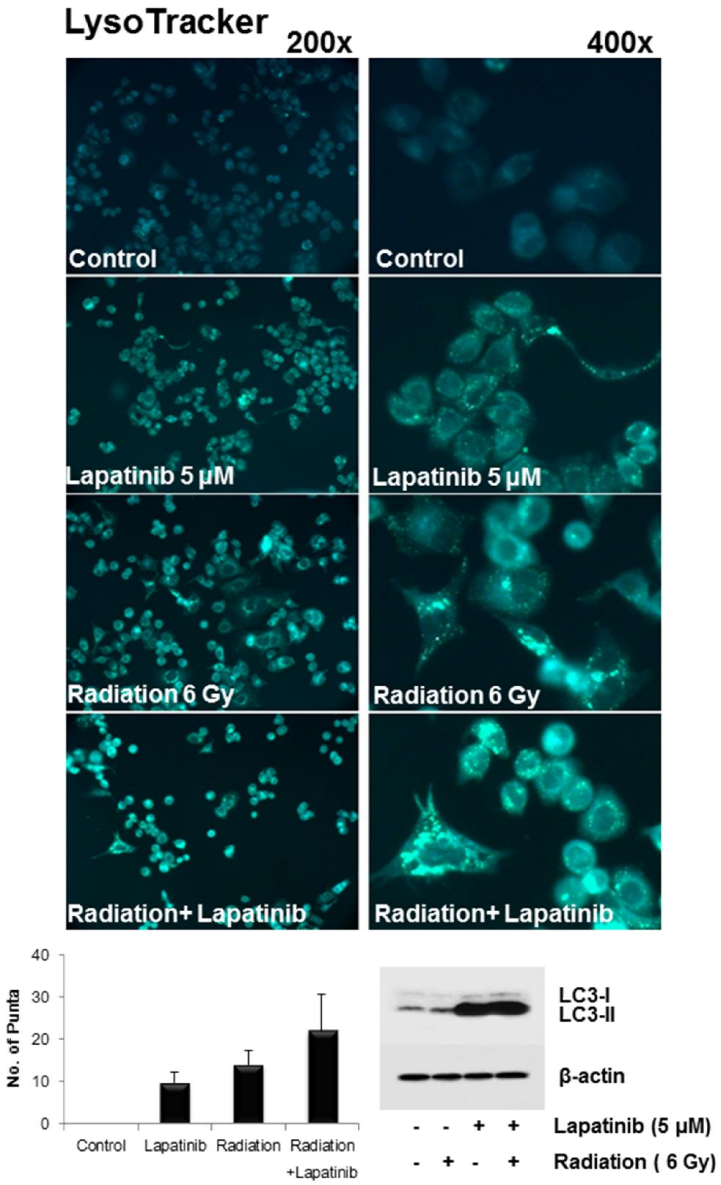
with PI and antibodies against annexin V. The number of apoptotic cells was determined by double stained foci with annexin V and PI. The number of apoptotic cells was highest in the cells treated with both lapatinib and radiation. The whole cell extracts were Western blotted with the indicated antibodies and the expression of caspase3 was increased when they are concurrently used.

(B)



(B) Senecence was examined by detecting the activity of β-galactosidase and notable change was observed in the cells treated with both radiation and lapatinib.

(C)



(C) SKBR cells were treated with lapatinib (5 μM), radiation (6 Gy), or lapatinib (5 μM) and radiation (6 Gy), then stained with LysoTracker® Green for 10 minutes. The number of Punta

formation in the cells treated with radiation and lapatinib was higher than any other cell groups, but it was not statistically significant compared to the cells with either lapatinib or radiation. The whole cell extracts were Western blotted with the indicated antibodies and the expression of LC3 was increased when lapatinib was used. $^{*}P \leq 0.05$. $^{***}P \leq 0.001$.

4. Autophagy does not significantly increase after the addition of lapatinib to cells treated with radiation alone.

To analyze autophagy, cells were stained with LysoTracker® Green for 10 minutes. Lysosomal localization was significantly higher in cells treated with lapatinib and radiation compared with the control, but there was no significant difference between cells treated with either lapatinib or radiation and those treated with both lapatinib and radiation (Figure 3C). Thus, based on this assay, in our hands, lapatinib does not potentiate radiation-induced autophagy.

5. Lapatinib does not affect radiosensitivity of normal human brain cells.

To examine the effect of lapatinib on normal human brain cells, NHAs treated with either DMSO or lapatinib were used. Lapatinib led down-regulated p-HER2 and p-EGFR (Figure 4). Lapatinib did not appear to increase radiosensitivity of NHAs in the clonogenic assay, and the SERs of the isoeffective dose at surviving fractions 0.5 and 0.05 were 1.0. Therefore, based on

this assay, lapatinib has little effect on human normal brain cells in contrast with breast cancer cell lines overexpressing HER2.

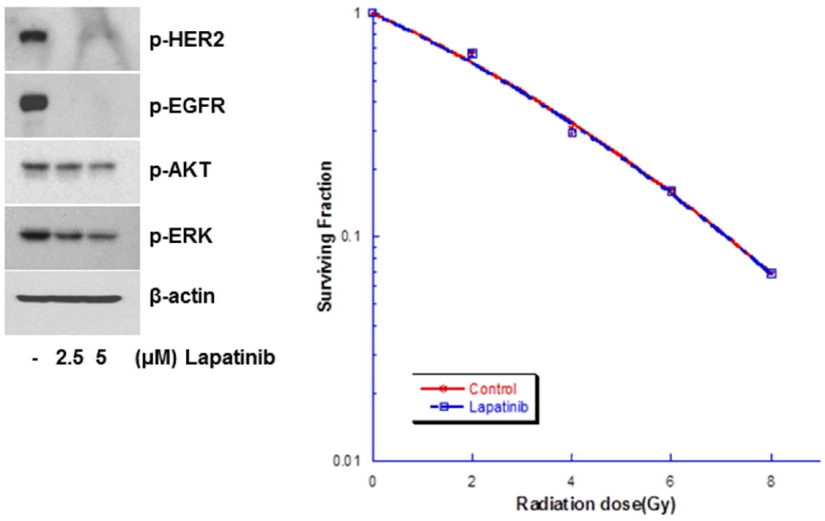


Figure 4. Lapatinib doesn't affect radiosensitivity of NHAs.

NHAs were treated with different concentration of lapatinib and the cell extracts were western blotted with the indicated antibodies. Lapatinib attenuated the expression of p-HER2 and p-EGFR in NHAs. The NHAs were treated with either DMSO or lapatinib and irradiated with 4-MV x-rays. Lapatinib did not appear to appreciably affect survival fraction of NHAs.

DISCUSSION

Lapatinib is expected to be used for breast cancer patients with brain metastasis because of its theoretical ability to cross the BBB resulting from its very low molecular weight (581 Da) (13). Lapatinib is the first HER2-targeting drug that has been identified in a preclinical study to have activity against the brain metastasis of breast cancer. When EGFR-overexpressing MDA-MB-231-BR (231-BR-HER2) brain-seeking breast cancer cells were injected in a mouse model, metastatic colonization in mouse brains was inhibited by 50-53% in response to lapatinib (22). Subsequently, concentrations of radioactively labeled lapatinib were validated in mice with 231-BR-HER2 brain-seeking breast cancer cells. The concentration found in the brain metastasis was 7-9 folds higher than in normal brain tissue; however, it was much lower than in peripheral metastasis (only 10-20% according to the time from lapatinib administration) (27).

Lapatinib alone for brain metastasis in patients with HER2-positive breast cancer demonstrated limited potential following prior RT as shown in Table 1 (14-20). Lin, *et al.* (14)

conducted a phase II trial for patients with HER2-positive breast cancer, brain metastasis, and prior trastuzumab treatment. The results were disappointing: no patients achieved a complete response (CR) and only one patient had a partial response (PR). In a subsequent expanded study with 242 patients, 15 patients (6%) achieved an objective response (defined as ≥50% volume reduction in brain metastases). For patients with disease progression on single-agent lapatinib, the option of additional capecitabine was allowed. In these patients, objective response was observed in 10 (20%) of 50 patients (15). Similarly, Sutherland, *et al.* (16) reported a response rate of 21% in 34 patients with brain metastasis who had been administered lapatinib and capecitabine. Since almost patients in these lapatinib trials underwent RT to the brain before enrollment, a potential delayed effect of RT may confuse the cytotoxic effect of lapatinib. In contrast, Bachelot, *et al.* (18) excluded patients previously treated with WBRT (whole brain radiotherapy), capecitabine, or lapatinib. Among 44 patients assessable for efficacy, 29 patients (66%) achieved ≥50% volume reduction in brain metastases after treatment with lapatinib and capecitabine.

Table 1. Prospective trials on lapatinib without brain radiotherapy for brain metastases

Authors	Phase	N	Prior Trastuzumab	Prior Radiotherapy	Regimen	Response (RECIST)	PFS, median	OS, median
de Azambuja, *et al.* (17)	I	18	All (L 8)	WBRT 14 WBRT+SRS 1	L+Tm	CR 0 (0%) PR 0 (0%)	2.6 M	10.9 M
Bachelot, *et al.* (18)	II	45	42	No	L+C	CR 2 (5%) PR 22 (52%) OR* 29 (66%)	TTP, 5.5 M	17 M
Iwata, *et al.* (19)	II	6	All	All	L	CR 0 PR* 1	Not reported	Not reported
Lin, *et al.* (20)	II	22	All	WBRT 13 SRS 1 WBRT 9 SRS 0	L+C (13) L+To (9)	CR 0 (0%) PR* 5 (38%) CR 0 (0%) PR* 0 (0%)	Not reported	Not reported
Sutherland, *et al.* (16)		34	All	WBRT (94%)	L+C	CR 1 (3%) PR 6 (18%)	TTP, 22 W	Not reported
Lin, *et al.* (15)	II	242	All	WBRT 229 SRS 64 WBRT+SRS 51	L (L+C 51) † 	CR 0 (0%) PR* 15 (6%) (PR* 11; 22%) †	2.4 M (3.7 M) †	6.4 M
Lin, *et al.* (14)	II	39	All	WBRT 20 SRS 6 WBRT+SRS 11	L	CR 0 (0%) PR 1 (3%)	TTP, 3 M	Not reported

* Partial response was defined as ≥50% volume reduction.

† Patients with disease progression on single-agent lapatinib were given the option to receive the combination of lapatinib plus capecitabine.

Abbreviations: L, lapatinib; Tm, temozolomide; C, capecitabine; To, topotecan; WBRT, whole brain radiotherapy; SRS, radiosurgery; RECIST, Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; OR, overall response; PFS, progression-free survival; TTP, time to progression; OS, overall survival; M, month; W, week.

Lapatinib has been shown to have a radiosensitizing effect in a preclinical breast cancer model. Sambade, *et al.* (23) reported *in vivo* data in which mice bearing xenografts of basal-like/EGFR-positive SUM149 and HER2-positive SUM225 breast cancer cells were treated with lapatinib and fractionated RT. The treatment with lapatinib alone had no influence on tumor growth for basal-like/EGFR+ SUM149 breast cancer tumors; however, it provided significant tumor volume reduction for HER2+ SUM225 breast cancer tumors. After the combination of lapatinib plus RT, mouse tumor volumes were significantly reduced in both the basal-like/EGFR+ SUM149 model and the HER2+ SUM225 model. During the study duration, treatment with both lapatinib and RT resulted in an average enhancement ratio of 1.25 for the HER2+ SUM225 model. According to immunohistochemical analyses, the radiosensitizing effect of lapatinib was associated with inhibition of AKT in the HER2+ SUM225 model. The radio-sensitizing effect of lapatinib was also demonstrated in our *in vitro* experiment. Furthermore, we could assume that lapatinib increases the radio-sensitivity by decreasing delayed repair of DNA damage via down-regulating non-homologous end-

joining repair, and increasing radiation-induced apoptosis and senescence.

There is only one clinical trial reporting the combination of lapatinib and WBRT, a phase I study for 35 patients with brain metastasis of HER2-positive breast cancer (28). Lapatinib 750mg was administered twice on the first day, followed by 1000, 1250, or 1500 mg once daily. WBRT (37.5 Gy in 15 fractions over three weeks) was started in the first eight days after the administration of lapatinib. During WBRT, patients received lapatinib continuously. After the completion of WBRT, patients were given 2 mg/kg of trastuzumab every week, combined with 1000 mg of lapatinib every day. Among 28 patients who had measurable brain lesions at baseline, the response rate was 79% (CR in three patients and PR in 19 patients) according to RECIST criteria. With a median follow-up time of 3.8 years, the median PFS and survival times were 4.8 and 19 months, respectively. Although, this study was designed to define the maximum tolerated dose of lapatinib and did not accomplish the primary goal due to toxicity, a high rate of CNS response was observed, indicating that lapatinib could be a good radiosensitizer in brain metastasis patients with

HER2-positive breast cancer.

Based on these *in vitro*, *in vivo* and clinical evidences supporting the radiosensitizing effect of lapatinib, several clinical trials are currently underway to elucidate the effect of lapatinib with radiation. The Radiation Therapy Oncology Group is performing a phase II randomized study on breast metastases in HER-positive breast cancer. Patients in the study are randomly assigned to receive WBRT with or without lapatinib. Lapatinib will be given 1000 mg orally once daily. Lapatinib will be started on the first day of WBRT and continue throughout WBRT and 21 days after the final day of WBRT without a drug holiday. The complete response rate in the brain will be assessed by a brain magnetic resonance imaging scan at 12 weeks post WBRT.

Recently, Stanford University initiated a phase II trial investigating the efficacy of lapatinib and RT in patients with locally advanced or locally recurrent breast cancer. Patients will receive lapatinib once daily starting seven days before RT until completion of RT. Response rates will be assessed after the treatment. The Hellenic Cooperative Oncology Group has

designed a phase II trial to evaluate the response rate of brain metastases from lung and breast tumors under treatment with WBRT and lapatinib. This study is a single-arm study and in which patients will be treated with WBRT (30 Gy in 10 fractions) and lapatinib 1250 mg once daily, followed by lapatinib 1500 mg once daily for a total of six weeks.

CONCLUSIONS

Lapatinib increases radiosensitivity of the HER2-postive breast cancer cells without effect to normal human astrocytes. Lapatinib hinders repair of DNA damage which probably relates with non-homologous end-joining repair. The cell killing effect of lapatinib may be related to increase radiation-induced apoptosis and senescence, rather than autophagy. At this time, several clinical trials using lapatinib concurrently with RT are ongoing.

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국문 초록

배경: 인간표피성장인자2(HER2)의 과발현은 유방암 환자의 20% 정도에서 발견된다. 트라스트주맙은 HER2 과발현 유방암에서 효과적인 치료법으로써 널리 이용되고 있지만, 혈액 뇌관문을 통과하지 못하는 제한점 때문에 오히려 뇌전이의 발생은 증가했다. 라파티닙은 분자량이 작아 혈액 뇌관문을 통과할 수 있어 전뇌방사선치료와 혼합하는 것이 HER2 과발현 유방암의 뇌전이 치료에 유망한 치료 전략이 될 것이다.

대상환자 및 방법: HER2 과발현된 유방암에서 라파티닙의 방사선 반응성에 대한 효과를 알아보기 위해, HER2/neu 유전자가 증폭되어 나타나는 유방암 세포인 SKBR3와 BT474에 라파티닙을 처리하여 세포주 실험을 시행하였다. 라파티닙의 방사선민감도를 알아보기 위해 클론원성 분석과 웨스턴블롯 분석을 이용하였고, DNA 손상을 감지하기 위해 γH2AX에 대한 항체를 이용하였다. 세포사 유형은 V-FITC/propidium iodide 이중염색과 β-galactosidase 염색, LysoTracker Green 염색을 통해 평가하였다.

결과: 라파티닙은 SKBR3와 BT474 세포의 p-HER2, p-EGFR, p-AKT, p-ERK의 발현을 감소시켰고, SKBR3와 BT474 세포의

방사선민감도를 증가시켰다 (SKBR3, 생존분율 0.5에서 SER 1.21; BT474, 생존분율 0.5에서 SER 1.26). 라파티닙은 방사선 유도하 γH2AX의 증가와 p-DNAPKc의 하향조절됨을 통해 DNA 손상의 수리를 저해하였음을 알 수 있었다. 또한 이러한 라파티닙과 방사선의 혼합으로 유도되는 세포사의 기전은 자가포식 보다는 방사선 유도 세포 자멸과 노화와 관계되었다. 라파티닙은 정상 인간 별아교세포의 방사선 손상은 증가시키지 않았다.

결론: 이러한 결과는 HER2 양성인 유방암 세포에 대한 방사선 민감도 효과를 보여주었고 향후 유방암 치료에 있어 방사선치료와 함께 라파티닙을 잠재적으로 응용해볼 수 있을 것이다.

주요어 : 유방암, 인간표피성장인자 2, 방사선치료, 라파티닙

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