Ionizing radiation is an important adjuvant therapy for breast cancer patients. Clinically, because damage to normal tissues surrounding tumor is unavoidable in radiotherapy, a high dose of IR is often fractionated to reduce the side-effects of radiotherapy. However, many studies have reported that IR paradoxically promotes the malignant glioma cell phenotypes, allowing relapse after treatment.\(^1\),\(^2\) Notably, this effect is frequently observed after exposure to fractionated radiation that is not sufficient to eradicate the primary tumor. In parallel, several strong lines of evidence have suggested that irradiation promotes invasiveness of cancer cells through induction of EMT. However, the molecular signaling mechanisms underlying radiation-induced EMT in breast cancer cells remain obscure.

The most widely studied member of the SFKs is SRC. As a non-receptor kinase, SRC is an integrator of divergent signal transduction pathways that regulate numerous cellular processes like cell proliferation, differentiation, migration, angiogenesis, and survival. Although transfection of normal fibroblasts with SRC alone does not induce cellular transformation,\(^3\) it is often elevated in multiple solid tumors.\(^4\)–\(^6\) In particular, SRC kinase is highly activated in human malignant breast cancer tissues compared with benign breast tumors or adjacent normal breast tissues, and this elevated SRC activity is correlated with poor metastasis-free survival.\(^7\)–\(^8\) In line with these observations, inhibition of SRC kinase effectively reduced the incidence of breast cancer metastasis and increased survival of mice,\(^9\) implicating SRC as a novel therapeutic target for the treatment of breast cancer cells.

In this study, we found that fractionated irradiation promotes migratory and invasive behavior of breast cancer cells through induction of the EMT program. Of note, irradiation caused activation of SRC which consequently activated PI3K, p38, and AKT, thereby promoting EMT through SLUG expression in breast cancer cells. Irradiation also increased resistance of cancer cells to anticancer treatments as well as the CD44^+ /CD24^- cell population known as CSCs in breast cancer. However, we show that downregulation of SRC suppresses CSCs as well as EMT that were induced by irradiation. In addition, targeting SRC sensitized breast cancer cells to anti-cancer drugs such as cisplatin, etoposide, paclitaxel, and IR. Taken together, our findings suggest that combining radiotherapy with targeting of SRC might attenuate the harmful effects of radiation and enhance the efficacy of breast cancer treatment.
radiation-induced EMT and enhance the efficacy of breast cancer treatment.

Materials and Methods

Chemical reagents and antibodies. Polyclonal antibody to CD44 (ab41478) was obtained from Abcam (Seoul, Korea). Polyclonal antibodies to AKT (9272S), p-AKT (S473) (9271S), p-AKT (T308) (9278S), SNAIL (3879), P38 (9121), and p-p38 (9218) were obtained from Cell Signaling Technology (Beverly, MA, USA). Polyclonal antibodies to vimentin (sc-5565), SLUG (SC-10436), TWH (SC-15395), c-SRC (sc-19), Lyn (sc-15), Fyn (sc-16), LCK (sc-15), and SOX2 (sc17319) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyclonal Antibody to ZEB1 (HPA027524) was purchased from Sigma (St, Louis, MO, USA). Polyclonal antibodies to N-cadherin (610920) and E-cadherin (612130) were purchased from BD Transduction Laboratory (Seoul, Korea). Monoclonal antibodies to β-actin, and DAPI were purchased from Sigma. Etoposide (341205), cisplatin (232120), and LY294002 a chemical inhibitor specific to PI3K (440202), were purchased from Calbiochem (San Diego, CA, USA). Paclitaxel (T912-SMG) was purchased from Sigma.

Cell culture. Human breast epithelial cell lines MCF7 and SKBR3 were purchased from ATCC (Manassas, VA, USA). Cells were cultured in a humidified 5% CO2 atmosphere at 37°C. The hormone receptor status of MCF7 cells is ER+, PR+, and HER2−. SKBR3 is ER− and HER2+. MCF7 cells were grown in minimum Eagle’s medium supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 g/mL). SKBR3 cells were grown in DMEM supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 g/mL). The DMEM, FBS, penicillin, streptomycin, and trypsin were purchased from Gibco (Seoul, Korea).

Invasion and migration assays. For invasion assays, MCF7 or SKBR3 cancer cells (1 × 104) were loaded in the upper well of a Transwell chamber (8-μm pore size; Corning Glass, Seoul, Korea) that was precoated with 10 mg/mL growth factor-reduced Matrigel (BD Biosciences, Seoul, Korea) on the upper side of the chamber with the lower well filled with 0.8 mL growth medium. After incubation for 48 h, non-invaded cells on the upper surface of the filter were removed with a cotton swab, and cells that had migrated onto the lower surface of the filter were fixed and stained with a Diff-Quick kit (Fisher, Pittsburgh, PA, USA) and photographed (magnification, ×20). Invasiveness was determined by counting cells in five microscopic fields per well, and the extent of invasion was expressed as an average number of cells per microscopic field. Cells were imaged by phase contrast microscopy (Leica Microsystems, Bannockburn, IL, USA). For the migration assay, we used the Transwell chambers with inserts that contained the same type of membrane but without the Matrigel coating.

Transfection. Cells were transfected with DN-p38 pCMV5, control empty vector pCMV5, or siRNA duplexes (40 nM) by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following the procedure recommended by the manufacturer. Western blot, immunocytochemistry, and FACS analysis or irradiation was carried out 48 h after transfection. All siRNAs targeting c-Src, AKT, and SLUG and a negative control siRNA were purchased from Genolution Pharmaceuticals (Seoul, Korea).

Western blot analysis. Cell lysates were prepared by incubating with lysis buffer (40 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.1% Nonidet-P40) supplemented with protease inhibitors. Proteins in whole-cell lysates were separated by SDS-PAGE and transferred to a nitrocellulose membrane (Amer sham, Arlington Heights, IL, USA). The membrane was blocked with 5% non-fat dry milk in TBS and incubated with primary antibodies overnight at 4°C. Blots were developed with HRP-conjugated secondary antibodies and proteins were visualized using ECL procedures (Amer sham), according to the manufacturer’s protocol. Secondary antibodies, anti-mouse IgG-HRP, anti-goat IgG-HRP, and anti-rabbit IgG-HRP were purchased from Santa Cruz Biotechnology.

Irradiation. Breast cancer cells were exposed to radiation using a 137Cs γ-ray source (Atomic Energy of Canada, Mississauga, Canada) at a dose rate of 3.81 Gy/min. Further analysis such as migration and invasion assay and Western blot and immunocytochemical analyses were carried out 48 h after fractionated irradiation (2 Gy × 3; 2 Gy/day for 3 days).

Immunocytochemistry. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS. Following fixation, cells were incubated at 4°C overnight with mouse polyclonal anti-human E-cadherin (1:200), rabbit polyclonal anti-human vimentin (1:200), PE-mouse anti-human CD44 (1:200), and Goat polyclonal anti-human anti-SLUG (1:200) primary antibodies, in 1% BSA with 0.1% Triton X-100. Immunostaining of proteins was visualized using Alexa Fluor 488-conjugated anti-rabbit and anti-mouse or anti-goat secondary antibodies (Molecular Probes, Seoul, Korea). Nuclei were counterstained with DAPI (Sigma). Immunostaining was observed on an Olympus IX71 fluorescence microscope (Olympus, Seoul, Korea).

Fluorescence-activated cell sorting analysis. Cells were stained with a PE-mouse anti-human CD44 (BD Pharmingen, Seoul, Korea) and FITC-mouse anti-human CD24 (BD Pharmingen) in PBS containing 0.5% BSA and 2 mM EDTA. As a control, cells were stained with PE mouse IgG1x isotype or FITC mouse IgG2a, κ isotype (BD Pharmingen). The CD44+/CD24− cells were then analyzed by FACS using a BD FACSCalibur system equipped with Cell Quest software (BD Biosciences).

Quantification of cell death. Cancer cells were treated with anticancer agents cisplatin (50 μM), etoposide (75 μM), paclitaxel (500 nM), or IR (10 Gy). At 48 h after treatment, cell death was measured by FACS analysis using propidium iodide staining. Cells were harvested by trypsinization, washed in PBS, and then incubated in propidium iodide (50 ng/mL) for 5 min at room temperature. Cells (10 000 per sample) were analyzed by BD FACSCalibur, using Cell Quest software.

Statistical analysis. All experimental data are reported as means; error bars represent SD. Statistical analyses were carried out using non-parametric Student’s t-tests.

Results

Irradiation promotes migratory and invasive properties of breast cancer cells through EMT. To study the harmful effects of radiotherapy, we examined whether IR causes breast cancer cells to acquire migratory and invasive properties. To this end, MCF7 and SKBR3 breast cancer cell lines were exposed to fractionated doses of radiation (2 Gy × 3; 2 Gy/day for 3 days). Cancer cells were then applied to migration and invasion assays that were carried out by counting migrated cells in Transwells that had been pre-coated with Matrigel (invasion assay) or left uncoated (migration assay). By these analysis, we observed that exposure to radiation enhances the migratory and invasive properties of breast cancer cells (Fig. 1a). As the
and invasion assay in MCF7 cancer cells transfected with siRNA targeting proteins (SRC, LYN, FYN, and LCK) using enolase as a substrate in MCF7 breast cancer cells after exposure to fractionated radiation. (b) Migration and invasion of MCF7 and SKBR3 breast cancer cells in Transwells after fractionated irradiation. (c) Western blot for EMT markers E-cadherin, N-cadherin, and vimentin in MCF7 and SKBR3 breast cancer cells after irradiation. (d) Western blot for EMT transcription factors SNAIL, SLUG, ZEB1, and TWIST in MCF7 and SKBR3 breast cancer cells after irradiation. β-actin was used as a loading control. Error bars represent mean ± SD of triplicate samples. **P < 0.01. Cont, control.

Fig. 2. Irradiation promotes epithelial–mesenchymal transition (EMT) through activation of SRC in breast cancer cells. (a) Kinase assay for SFK proteins (SRC, LYN, FYN, and LCK) using enolase as a substrate in MCF7 breast cancer cells after exposure to fractionated radiation. (b) Migration and invasion assay in MCF7 cancer cells transfected with siRNA targeting SRC or scrambled control siRNA (si-Cont) prior to irradiation. (c) Western blot analysis (d) and immunocytochemistry for EMT markers E-cadherin, N-cadherin, and vimentin in MCF7 cancer cells transfected with siRNA targeting SRC or scrambled control siRNA prior to irradiation. (e, f) Western blot analysis for EMT transcription factors SLUG, SNAIL, ZEB1, and TWIST (e), and immunocytochemistry for EMT transcription factor SLUG (f) in MCF7 cancer cells transfected with siRNA targeting SRC or scrambled control siRNA prior to irradiation. (g, h) Migration and invasion assay in MCF7 (g) and SKBR3 (h) cancer cells transfected with SRC WT, mutant form SRC Y527F, or control vector pcDNA3.1. (i) Western blot analysis for E-cadherin and N-cadherin in MCF7 cells transfected with SRC WT, mutant form SRC Y527F, or control vector pcDNA3.1. β-actin was used as a loading control. Error bars represent mean ± SD of triplicate samples. *P < 0.05; **P < 0.01. Cont, control; IP, Immunoprecipitation.

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results suggest that radiation triggers the EMT program through activation of SRC in breast cancer cells.

**Radiation-activated SRC transduces intracellular signaling pathways PI3K/AKT and p38 MAPK to increase migratory and invasive behavior.** In the above data, we showed that irradiation induces activation of SRC, thereby increasing SLUG expression and triggering EMT in breast cancer cells. Thus, we examined the intracellular signaling pathways that are triggered by SRC and are responsible for radiation-induced EMT in breast cancer cells. Importantly, we found that PI3K/AKT and p38 MAPK pathways are activated by irradiation; however, treatment with siRNA targeting SRC attenuated the radiation-induced activation of PI3K/AKT and p38 (Fig. 3a). We next examined the phosphorylation status of AKT after transfection with the DN mutant form of p38. Of note, transfection with DN-p38 effectively inhibited the phosphorylation of AKT on Ser473, whereas DN-p38 had no effect on the phosphorylation of Thr308, suggesting that p38 is a downstream effector of SRC, promoting phosphorylation of AKT on Ser473 (Fig. 3b). Also, transfection with DN-p38 attenuated radiation-induced EMT markers such as N-cadherin and vimentin, and EMT transcription factor SLUG (Fig. S2b). In line with these results, treatment with siRNA targeting AKT effectively mitigated the radiation-induced migratory and invasive properties of MCF7 breast cancer cells (Fig. 3c). In agreement, treatment with PI3K specific inhibitor LY294002 attenuated radiation-induced EMT markers and regulator SLUG (Fig. S2c). Taken together, these results suggest that radiation promotes the EMT program by SRC-mediated activation of PI3K and p38 MAPK that consequently activates AKT signaling in breast cancer cells.

**Radiation-activated SRC promotes CSCs.** As EMT is often associated with the CSC population, we examined whether radiation-activated SRC is also involved in expansion of the CSC population. To this end, we analyzed the CD44+/CD24− cell population in both MCF7 and SKBR3 breast cancer cells after exposure to a single dose (6 Gy) or fractionated dose (2 Gy × 3) of radiation. Notably, irradiation caused an increase in the CD44+/CD24− cell population, well-known as CSCs in breast cancer (Fig. 4a). Intriguingly, fractionated irradiation had more effect on the increase of CD44+/CD24− cells compared to single irradiation, although irradiated doses were equal. However, treatment with siRNA targeting SRC suppressed the radiation-induced expansion of CD44− cells to basal levels (Fig. 4b).

Much evidence has suggested that SOX2 and Notch are also critical proteins in the maintenance of breast CSCs.10-12 To further confirm that radiation-activated SRC promotes breast CSCs, we examined the expression levels of SOX2, Notch-1, and Notch-2 as well as CD44 in MCF7 cells after irradiation and treatment with siRNA targeting SRC. In agreement with the above data, irradiation increased the expression of CD44, SOX2, and Notch-2; however, treatment with siRNA against SRC attenuated these expression levels that were analyzed by Western blot and immunocytochemistry (Fig. 4c,d).

Because radiation-activated SRC promoted EMT through activation of AKT, we next examined whether radiation-activated SRC increases the CSC population through AKT. As expected, downregulation of AKT abolished the radiation-induced expansion of the CD44+ cell population (Fig. 4e). In parallel, downregulation of p38 that was activated by radiation-induced SRC also decreased CD44+ cells (Fig. 4f). To further confirm that radiation-activated SRC increases CSCs, we overexpressed SRC and analyzed CD44+ cells. Consistent with the effect of SRC downregulation, overexpression of either WT SRC or mutant form Y527F caused an increase in CD44+ cells (Fig. 4g). The protein level of CD44 was also increased by overexpression of SRC WT or SRC Y527F (Fig. 4h). Collectively, these results suggest that radiation-activated SRC promotes CSCs as well as EMT in breast cancer cells.

**Radiation-activated SRC confers resistance to anticancer agents on breast cancer cells.** As CSCs are reported to be resistant to chemo- and radiotherapy,13,14 we next examined whether irradiation increases the resistance of cancer cells to anticancer treatments through activation of SRC. To this end, breast cancer cells were exposed to fractionated radiation, and on the following day cells were treated with anticancer agents cisplatin, etoposide, or paclitaxel. When cell death was analyzed by FACS, we found that breast cancer cells exposed to fractionated irradiation increased by overexpression of SRC WT or SRC Y527F (Fig. 4h). Consistently, these results suggest that radiation-activated SRC increases SRC abundance and confers resistance to anticancer agents on breast cancer cells.

Fig. 3. Radiation-activated SRC transduces intracellular signaling of phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and p38 MAPK to increase migratory and invasive behavior. (a) PI3 kinase assay and Western blot analysis for phosphorylation status of AKT and p38 MAPK in MCF7 cancer cells transfected with siRNA targeting SRC or scrambled control siRNA (si-Cont) prior to irradiation. (b) Western blot analysis for phosphorylation status of AKT in MCF7 cancer cells transfected by dominant negative (DN)-p38 or control pCMV5 prior to irradiation. (c) Migration and invasion assay in MCF7 breast cancer cells transfected with siRNA targeting AKT (si-AKT) or scrambled control siRNA prior to irradiation. (d) Western blot analysis of the phosphorylation status of AKT in MCF7 cancer cells transfected by dominant negative (DN)-p38 or control pCMV5 prior to irradiation. Error bars represent mean ± SD of triplicate samples. **P < 0.01. PTEN, phosphatase and tensin homolog. Cont, control.
Radiation showed more resistance to cisplatin, etoposide, and paclitaxel compared to non-irradiated cells (Fig. 5a–c). To examine whether pretreatment with fractionated radiation also confers radio-resistance on cancer cells, MCF7 cells were exposed to fractionated radiation and followed exposure to IR (10 Gy). Consistently, we observed that pretreatment with fractionated irradiation caused breast cancer cells to become more resistant to IR (10 Gy), compared to non-irradiated control cells (Fig. 5d). However, pretreatment with siRNA targeting SRC prevented breast cancer cells from acquiring radiation-induced resistance to cisplatin, etoposide, paclitaxel, and IR (Fig. 5). Taken together, these results suggest that radiation-activated SRC is necessary for the acquisition of resistance to chemo- and radiotherapy in breast cancer cells.

**Discussion**

Despite the fact that radiotherapy exerts its therapeutic effect by inducing apoptosis of tumor cells, emerging clinical evidence suggests that irradiation promotes the metastatic behavior of many cancers, including breast cancer.\(^1\) This rapid, accumulating evidence suggests that IR promotes cancer metastatic ability by triggering EMT that has a central role in cancer metastasis and has become the subject of intense study.
However, the signaling molecular mechanisms underlying radiation-induced EMT remain obscure.

In this study, we found that irradiation promotes the metastatic ability of breast cancer cells through EMT. Exposure to fractionated radiation increased expression of mesenchymal markers such as N-cadherin and vimentin, whereas it decreased epithelial marker E-cadherin. In parallel, irradiation induced EMT transcription factor SLUG but not other factors SNAIL, ZEB1, and TWIST, suggesting that radiation promotes EMT in breast cancer cells through SLUG induction.

Very recently, several lines of evidence suggested that EMT is closely associated with acquisition of CSC phenotypes and resistance to anticancer agents, indicating that EMT is a complex cellular program driving to the multifaceted cancer progression. In agreement with these previous reports, we found that radiation-induced EMT was accompanied with an increase of CSCs. Also, irradiation caused breast cancer cells to acquire resistance to anticancer agents such as cisplatin, etoposide, and paclitaxel as well as IR. However, knockdown of SRC, which was activated by irradiation, efficiently attenuated the radiation-induced EMT, expansion of the CSC population, and resistance to anticancer agents, indicating that SRC is a universal signal node, and targeting of SRC could overcome multiple mechanisms of resistance against anticancer agents.

In this study, we observed that radiation-activated SRC promoted malignant phenotypes of breast cancer cells through the PI3K/AKT and p38 signaling pathways. We showed that downregulation of SRC mitigates radiation-enhanced resistance of breast cancer cells to anticancer agents. Quantification of cell death by FACS analysis using propidium iodide staining. MCF7 breast cancer cells transfected with siRNA targeting SRC (si-SRC) or scrambled control siRNA (si-Cont) were exposed to fractionated radiation and then treated with cisplatin (50 µM) (a), etoposide (b), paclitaxel (500 nM) (c), or exposure to ionizing radiation (10 Gy) (d). **P < 0.01. Cont, control; N.S., Not significant.

In summary, our findings suggest that radiation promotes the EMT program by SRC-mediated activation of PI3K and p38 MAPK that consequently activates AKT signaling in breast cancer cells.
cancer cells. These findings imply that the targeting of SRC might mitigate radiation-induced malignant phenotypes and increase the efficacy of radiotherapy for breast cancer treatment.

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Disclosure Statement

The authors have no conflict of interest.

References

1. Durks PB. Brain tumor stem cells: bringing order to the chaos of brain cancer. J Clin Oncol 2008; 26: 2916–24.
2. Squatrito M, Brennan CW, Helmy K, Huse JT, Petriji RH, Holland EC. Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas. Cancer Cell 2010; 18: 619–29.
3. Shalloway D, Coussens PM, Yaciuk P. Overexpression of the c-src protein does not induce transformation of NIH 3T3 cells. Proc Natl Acad Sci U S A 1984; 81: 7071–5.
4. Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. Cancer Metastasis Rev 2003; 22: 337–58.
5. Yeatman TJ. A renaissance for SRC. Nat Rev Cancer 2004; 4: 470–80.
6. Jacobs C, Rubsamen H. Expression of pp60c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. Cancer Res 1983; 43: 1696–702.
7. Hennipman A, van Oirschot BA, Smits J, Rijksen G, Staal GE. Tyrosine kinase activity in breast cancer, benign breast disease, and normal breast tissue. Cancer Res 1989; 49: 516–21.
8. Verbeek BS, Vroom TM, Adriaenssen-Slot SS, et al. c-Src protein expression is increased in human breast cancer. An immunohistochemical and biochemical analysis. J Pathol 1996; 180: 383–8.
9. Rucci N, Recchia I, Angelucci A, et al. Inhibition of protein kinase c-Src reduces the incidence of breast cancer metastases and increases survival in mice: implications for therapy. J Pharmacol Exp Ther 2006; 318: 161–72.
10. Leis O, Eguara A, Lopez-Arrihilaga E, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene 2012; 31: 1334–65.
11. Lagadec C, Vlashi E, Alhiyari Y, Phillips TM, Bohkur Dratver M, Pajonk F. Radiation-induced Notch signaling in breast cancer stem cells. Int J Radiat Oncol Biol Phys 2013; 87: 609–18.
12. Farme G, Clarke RB. Mammary stem cells and breast cancer–role of Notch signalling. Stem Cell Rev 2007; 3: 169–75.
13. Zhang M, Atkinson RL, Rosen JM. Selective targeting of radiation-resistant tumor-initiating cells. Proc Natl Acad Sci U S A 2010; 107: 3522–7.
14. Frosina G. DNA repair and resistance of gliomas to chemotherapy and radiotherapy. Mol Cancer Res 2009; 7: 989–99.
15. Kawamoto A, Yokoe T, Tanaka K, et al. Radiation induces epithelial-mesenchymal transition in colorectal cancer cells. Oncol Rep 2012; 27: 51–7.
16. Yan S, Wang Y, Yang Q, et al. Low-dose radiation-induced epithelial-mesenchymal transition through NF-kappaB in cervical cancer cells. Int J Oncol 2013; 42: 1801–6.
17. Liu W, Huang YJ, Liu C, et al. Inhibition of TBK1 attenuates radiation-induced epithelial-mesenchymal transition of A549 human lung cancer cells via activation of GSK-3beta and repression of ZEB1. Lab Invest 2014; 94: 362–70.
18. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704–15.
19. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010; 29: 4741–51.
20. Tryfonopoulos D, Walsh S, Collins DM, et al. Src: a potential target for the treatment of triple-negative breast cancer. Ann Oncol 2011; 22: 2334–40.
21. Zhang S, Huang WC, Li P, et al. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. Nat Med 2011; 17: 461–9.
22. Thobe BM, Frink M, Choudhry MA, Schwacha MG, Bland KI, Chaudry IH. Src family kinases regulate p38 MAPK-mediated IL-6 production in Kupffer cells following hypoxia. Am J Physiol Cell Physiol 2006; 291: C476–82.
23. Kim MJ, Byun JY, Yun CH, Park IC, Lee KH, Lee SJ. c-Src-p38 mitogen-activated protein kinase signaling is required for Akt activation in response to ionizing radiation. Mol Cancer Res 2008; 6: 1872–80.
24. Siva S, MacManus M, Kron T, et al. A pattern of early radiation-induced inflammatory cytokine expression is associated with lung toxicity in patients with non-small cell lung cancer. PLoS ONE 2014; 9: e109560.
25. Muller K, Meinke V. Radiation-induced alterations in cytokine production by skin cells. Exp Hematol 2007; 35: 96–104.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Ionizing radiation promotes epithelial–mesenchymal transition in breast cancer cells through SLUG.

Fig. S2. Overexpression of SRC and radiation-induced epithelial–mesenchymal transition by phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and p38 MAPK, downstream effectors of SRC in breast cancer cells.

Abbreviations

AKT protein kinase B
CSC cancer stem cell
DN dominant negative
EMT epithelial–mesenchymal transition
ER estrogen receptor
HER2 human epidermal growth factor receptor 2
IR ionizing radiation
p- phosphorylated
PE phycoerythrin
PI3K phosphatidylinositol 3-kinase
PR progesterone receptor
SFK SRC family kinase