Pyrotinib enhances the radiosensitivity of HER2-overexpressing gastric and breast cancer cells

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Abstract. The overexpression or amplification of HER2 has been observed in a significant proportion of both gastric cancer (GC) and breast cancer (BC) cases. Pyrotinib is an irreversible dual (EGFR/HER2) tyrosine kinase inhibitor (TKI), newly evaluated for the treatment of HER2-overexpressing cancer types. As radiotherapy (RT) serves a crucial role in controlling the local recurrence of GC and BC, the present study investigated the impact of pyrotinib on the irradiation response. The current results demonstrated that pyrotinib enhanced the radiosensitivity of HER2-overexpressing GC and BC cells in vitro and in vivo. In both NCI-N87 and SKBR3 cells, pyrotinib suppressed the irradiation-induced HER2 nuclear transport. Furthermore, pyrotinib increased DNA damage induced by irradiation in both cancer cell lines. Pyrotinib also enhanced the cytotoxicity of docetaxel, which may provide a novel strategy for potential drug combinations. Thus, pyrotinib is a promising irradiation sensitizer in patients with HER2-overexpressing GC and BC. The present results provide a theoretical foundation for further clinical evaluation of pyrotinib.

Introduction

Gastric cancer (GC) and breast cancer (BC) are two of the most common malignant cancer types worldwide, particularly in China. In 2015, GC caused ~498,000 mortalities and 679,000 new cases (1). Among women ≤45 years of age, BC remains the leading cause of cancer-related mortality, followed by lung cancer (1). Due to cancer recurrence and metastasis, numerous patients fail treatment despite improvements in GC and BC therapy. In patients with GC and BC, radiotherapy (RT) serves a crucial role in controlling local recurrence (2). In the past decades, great progress has been made to improve the ability to select appropriate patients, and thus, maximize clinical benefits, while minimizing toxicity and disease burden (2). However, limitations persist despite the appropriate use of RT. Intrinsic or acquired radioresistance and non-specific toxicity limit the efficacy of RT (2). To overcome these shortcomings, increased efforts have been made to discover an effective radiosensitizer characterized by higher efficacy and lower toxicity.

HER2 is upregulated in 13-23% of GC and 15-30% of BC cases (3-5). HER2 acts as an oncogene in different cancer types, most likely because amplification of this gene consistently results in HER2 upregulation and the acquisition of beneficial properties of malignant cells (6). Moreover, accumulating evidence has revealed that HER2 is involved in radioresistance (7,8). Previous studies have reported that HER2-targeting agents, including trastuzumab, lapatinib and afatinib, could be utilized to sensitize HER2-overexpressing cancer cells to RT by inhibiting signaling pathways inducing radioresistance (9-11). The combination of RT and trastuzumab has been applied in clinical practice and has been investigated mainly as an adjuvant therapy (12). The side effects observed with the concurrent use of trastuzumab and locoregional RT in BC are acceptable, demonstrating satisfactory outcomes (12); however, longer follow-ups are required to further identify these effects. The mechanism of HER2 induced-radioresistance in cancer cells also remains unknown. Previous studies have revealed that HER2 transport from membranes to nuclei contributes to radioresistance, and the survival of irradiated HER2-overexpressing cancer cells is decreased after inhibiting this pathway (13,14). Not all patients with HER2 overexpression benefit from trastuzumab. For instance, certain patients develop resistance to trastuzumab after 1 year, even those who achieved an initial reaction to this drug (15). Similarly, primary and acquired resistance to lapatinib, a reversible dual tyrosine kinase inhibitor (TKI) of EGFR and HER2, remains a significant clinical
problem (16). To further enhance HER2 inhibition, pyrotinib, a novel irreversible dual (EGFR/HER2) TKI, has been evaluated for the treatment of HER2-overexpressing cancer types. Compared with trastuzumab and lapatinib, pyrotinib effectively overcomes drug resistance triggered by EGFR or HER2 mutations, which is due to the covalent binding between cysteine residues of the receptor and electrophilic groups of pyrotinib (17,18). Currently, enhancing the sensitivity of cancer cells to irradiation by targeting the HER family has been approved as a novel method to potentiate the therapeutic efficacy of irradiation (19). Although the antitumor effect of pyrotinib has been previously reported (20), the role of pyrotinib in sensitizing HER2-overexpressing cancer cells to irradiation needs to be further elucidated.

The present study hypothesized that pyrotinib may be a promising irradiation sensitizer in patients with HER2-overexpressing GC and BC. The current study aimed to investigate the radiosensitizing effect of pyrotinib in HER2-overexpressing GC and BC cell lines, as well as in xenograft models. Additionally, the potential mechanism involving HER2 nuclear transport was identified.

Materials and methods

Cell culture and reagents. Human GC cell lines (NCI-N87 and MKN28) and human BC cell lines (SKBR3 and MCF7) were purchased from the Type Culture Collection of the Chinese Academy of Sciences. All cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. All cell lines were authenticated using Short Tandem Repeat profiles. Pyrotinib was gifted by Hengrui Medicine Co., Ltd. The cytotoxic drugs fluorouracil, cisplatin, docetaxel and epirubicin were obtained from Sigma-Aldrich (Merck KGaA). All drugs were dissolved in DMSO at appropriate concentrations (pyrotinib at 100 mg/ml, fluorouracil at 100 mM, cisplatin at 10 µM, docetaxel at 100 µM and epirubicin at 10 µM).

X irradiation. The irradiation of cancer cells and mice was performed using an RS2000 X-ray Biological Research Irradiator (160 kV; 25 mA; 3-mm copper filter; Rad Source Technologies, Inc.).

Western blotting. After treatment, the whole-cell protein was extracted by lysing cells with RIPA buffer, which was supplied with a complete protease inhibitor cocktail (Roche Diagnostics, Inc.). Nuclear protein was extracted using the Nuclear and Cytoplasmic Protein Extraction kit (cat. no. P0028; Beyotime Institute of Biotechnology) following the manufacturer's instructions, at 0, 0.25, 0.5, 1 and 6 h after irradiation. The Enhanced BCA Protein Assay kit (cat. no. P0010; Beyotime Institute of Biotechnology) was used to determine protein concentrations. An equal amount of protein (20 µg) was electrophoresed in 8-10% SDS-PAGE and then transferred to the PVDF membrane (cat. no. IPVH00010; EMD Millipore). After blocking with 5% non-fat milk at room temperature for 10-20 min at room temperature. After washing in PBS, the membranes were incubated with the secondary antibody (1:5,000; cat. no. A0208; Beyotime Institute of Biotechnology) labeled with horseradish peroxidase for 1 h at 37°C. The blots were visualized using the Enhanced Chemiluminescence Detection kit (cat. no. 32209; Thermo Fisher Scientific, Inc.).

Immunofluorescence. Cancer cells (5x10⁴ cells/ml) were cultured on slips and fixed with 4% paraformaldehyde for 10-20 min at room temperature. After washing in PBS, the cells were permeabilized with 0.1% Triton X-100, blocked using 5% BSA (Gibco; Thermo Fisher Scientific, Inc.) for 1 h at room temperature and incubated with primary antibodies overnight at 4°C. Primary antibodies used were as follows: Anti-HER2 (1:200; cat. no. 2165; Cell Signaling Technology, Inc.) and γ-H2AX (1:200; cat. no. 9718; Cell Signaling Technology, Inc.). After washing in PBS three times, the cells were incubated with FITC-conjugated anti-rabbit secondary antibody (1:500; cat. no. BA1105; Wuhan Boster Biological Technology, Ltd.) for 1 h at room temperature in the dark. DAPI stained for 3 min at room temperature and was used for nuclear localization, as well as to assess the quality of the experiment. The slides were washed with PBS until the excess DAPI was removed. After drying, the slides were sealed with coverslips and mounting medium. Finally, the cells were visualized with fluorescent microscope (magnification, x200).

Cell viability assay. To assess cell viability, cells were seeded in 96-well plates at a density of 5,000 cells per well and cultured overnight to grow adhering to the wall. Then, the cells were treated with the indicated concentrations of drugs and/or the indicated dose of irradiation at 37°C. An increasing dose of pyrotinib (0, 0.1, 1, 10, 100 and 1,000 µg/ml) was used to examine the sensitivity of GC and BC cells to pyrotinib. After 48 h, the original culture media were removed. The cells of each well were washed with PBS and maintained in fresh culture media with 10% Cell Counting Kit-8 reagent (CCK-8; cat. no. AR1160; Wuhan Boster Biological Technology, Ltd.) for another 1 h at 37°C according to the manufacturer's instructions. The absorbance of viable cells was measured using a microplate reader at 450 nm. Each treatment was performed in ≥3 replicate wells.

Clonogenic survival assay. Cancer cells were cultured in 6-well plates at different densities (100, 200, 600, 2,000 and 6,000 cells/well). Following pretreatment with pyrotinib (0.1 µg/ml, 37°C for 24 h), cells were exposed to irradiation at different radiation doses (0, 2, 4, 6 and 8 Gy). After incubation for 14-20 days, cells were fixed with anhydrous methanol for 15 min at room temperature and stained with 0.1% crystal violet for 20-30 min at room temperature. Colonies counting >50 cells were considered as surviving clones. The plating efficiency (PE) and surviving fraction (SF) were calculated as follows: PE=mean colony number in
un-irradiated controls/number of seeded cells. SF=mean colony number/number of cells seeded x PE. A Multi-target single-hit model \( \text{SF}=1-(1-e^{-\text{D}/D_0})^N \) was used to calculate \( D_0 \) (the average irradiation dose of lethal exposure), with the sensitizing enhancement ratio (SER) determined as follows, \( \text{SER}=D_0 \) of combination treatment/\( D_0 \) of irradiation treatment alone.

**Measurement of apoptosis.** Quantification of cell apoptosis was performed using the Annexin V-FITC/PI Apoptosis Detection kit (cat. no. KGA105; Nanjing KeyGen Biotech Co., Ltd.) following the manufacturer's protocol. After treatment, cells were trypsinized before collection and suspended in 300 µl binding buffer. Then, 5 µl Annexin V-FITC and 5 µl PI were added to each sample. The cell samples were incubated in the dark for 15 min at room temperature, detected using flow cytometry (BD LSRFortessa; BD Biosciences), and analyzed using FlowJo software version 10.7 (FlowJo LLC). The apoptotic rate was calculated as the percentage of early + late apoptotic cells.

**Cell cycle distribution.** Cell cycle distribution was detected using the PI/RNase buffer (cat. no. KGA512; Nanjing KeyGen Biotech Co., Ltd.) following the manufacturer's protocol. NCI-N87 and SKBR3 cells were treated with pyrotinib (0.1 µg/ml), irradiation (4 Gy) or the combination of pyrotinib and irradiation. After treatment, cells were harvested via trypsinization, washed with PBS and fixed with 70% ethanol for 20 min at 4°C. Then, cell samples were suspended in RNase buffer with PI, shielded from light for 30 min at room temperature and analyzed via flow cytometry (BD LSRFortessa; BD Biosciences) and FlowJo software version 10.7 (FlowJo LLC).

**Xenograft models.** Animal experiments were performed according to the guidelines of, and were approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (permit no. TJ2015A). Female nude (BALB/c nu-nu; age, 4–6 weeks; weight, 12–15 g) mice were obtained from Charles River, Ltd., and fed under pathogen-free conditions (temperature 26‑28˚C; humidity, 40‑60%; 10 h light/14 h dark cycle; permit no. TJ2015A). Female nude (BALB/c nu-nu; age, 4–6 weeks; weight, 12‑15 g) mice were obtained from Charles River, Ltd., and fed under pathogen-free conditions (temperature 26‑28˚C; humidity, 40‑60%; 10 h light/14 h dark cycle; permit no. TJ2015A). Female nude (BALB/c nu-nu; age, 4–6 weeks; weight, 12‑15 g) mice were obtained from Charles River, Ltd., and fed under pathogen-free conditions (temperature 26‑28˚C; humidity, 40‑60%; 10 h light/14 h dark cycle; permit no. TJ2015A).

HER2-overexpressing GC and BC cells are selectively sensitive to pyrotinib inhibition. To identify HER2-overexpressing GC and BC cells, the degree of HER2 protein expression was evaluated in two GC cell lines (NCI-N87 and MKN28) and two BC cell lines (SKBR3 and MCF-7). Western blotting results demonstrated that NCI-N87 and SKBR3 cells had upregulated HER2 protein expression, while the remaining cell lines did not overexpress HER2 protein (Fig. 1A). Furthermore, it was observed that HER2 was mainly located on cell membranes (Fig. 1B).

To examine the sensitivity of GC and BC cells to pyrotinib, each cell line was exposed to increasing doses of pyrotinib. Compared with HER2 non-overexpressing GC and BC cells, NCI-N87 and SKBR3 cells displayed sensitivity to pyrotinib (Fig. 1C).

Pyrotinib enhances the radiosensitivity of HER2-overexpressing GC and BC cells. After treatment for 48 h, pyrotinib induced the dose-dependent proliferative inhibition of NCI-N87 and SKBR3 cells (Fig. 1C). Pyrotinib at 0.1 µg/ml was unable to suppress cell viability significantly, and the survival rate of cancer cells was >80%. Therefore, the drug concentration of 0.1 µg/ml was selected for further in vitro experiments. The combination of irradiation and pyrotinib significantly inhibited proliferation (Fig. 2A) and clonogenic survival in NCI-N87 and SKBR3 cells (Fig. 2B). Compared with cells treated with irradiation alone, pretreatment of pyrotinib decreased colony formation of cancer cells after irradiation, suggesting that pyrotinib enhanced the radiosensitivity of NCI-N87 and SKBR3 cells (SER=1.375 and 1.326; Fig. 2B).

As pyrotinib sensitized HER2-overexpressing GC and BC cells to irradiation, this drug may function as a promising radiosensitizer in indicated patients, and its targeted toxicity toward HER2-overexpressing cancer cells may augment the therapeutic effect.

**Pyrotinib augments irradiation response in HER2-overexpressing tumor xenografts.** Based on the in vitro radiosensitizing effects in HER2-overexpressing cancer cells,
the potential irradiation response of pyrotinib was further examined in vivo. NCI-N87 and SKBR3 cells were used to perform tumor xenograft experiments in athymic nude mice. Following the establishment of the xenograft models,
different groups of mice were treated with a single-dose of irradiation (10 Gy) alone, pyrotinib (10 mg/kg/day) alone or a combination of irradiation and pyrotinib. The antitumor effect of these treatments was determined by measuring the tumor volume (Fig. 3). In both NCI-N87 and SKBR3 xenograft tumors, irradiation combined with pyrotinib demonstrated a greater antitumor effect compared with irradiation alone, although treatment with irradiation or pyrotinib alone treatment also had a significant inhibitory effect on tumor growth. Moreover, the combination strategy did not induce any mortality or a significant decrease in mouse body weight (data not shown), suggesting that pyrotinib may act as an effective and safe RT sensitizer.

Pyrotinib inhibits irradiation-induced HER2 nuclear transport. Following HER2 activation, two main cell signaling pathways, including PI3K/Akt and MEK/MAPK pathways, could be activated involving in the mitogenic and survival signals (7). To assess their involvement in pyrotinib-mediated radiosensitization in the HER2-overexpressing GC and BC cells, the expression levels of p-Akt and MAPK were examined after irradiation, with or without the pretreatment of pyrotinib. Western blotting results demonstrated that pyrotinib markedly suppressed Akt signaling and exerted no effect on MAPK signaling in irradiated SKBR3 cells. In NCI-N87 cells, pretreatment of pyrotinib before irradiation presented no notable influences on Akt and MAPK signaling (Fig. 4A).

Previous studies have reported that the nuclear transport pathway also contributes to radioresistance, especially HER2 transport from membranes to nuclei (13,14). To examine the influence of pyrotinib on irradiation-induced HER2-nuclear transport, the nuclear protein was extracted at 0, 0.25, 0.5, 1 and 6 h after irradiation, with or without pretreatment of pyrotinib. The expression of HER2 in nuclear fraction was notably increased after irradiation, attaining a peak at 0.5 h after irradiation. In NCI-N87 and SKBR3 cells, treatment with pyrotinib before irradiation decreased HER2 expression in the nucleus at different time-points (Fig. 4B). Thus, it was indicated that pyrotinib can decrease the irradiation-induced HER2 nuclear transport, improving the radiosensitivity of HER2-overexpressing cells.

Pyrotinib increases apoptosis and G2/M arrest induced by irradiation in NCI-N87 cells. To determine whether pyrotinib affected radiosensitivity by inducing apoptosis, flow cytometric analysis was performed. The irradiation dose of 4 Gy was selected, since the irradiation of 4 Gy combined with pyrotinib suppressed cell viability significantly and the survival rate of cancer cells was modest (Fig. 2A). To evaluate the occurrence of apoptosis, NCI-N87 and SKBR3 cells were

Figure 3. Pyrotinib augments irradiation response in HER2-overexpressing tumor xenografts. Nude mice bearing NCI-N87 or SKBR3 xenografts were randomly divided into four groups: Control group, mice treated with pyrotinib alone, irradiation alone or a combination of pyrotinib and irradiation. (A) Tumor volume was measured thrice weekly for 21 days. (B) Tumors were dissected 21 days after treatment. Representative external appearances of tumor and tumor-bearing mice are presented. (C) Representative images of hematoxylin and eosin staining of xenografts (magnification, x200). Data are presented as the mean ± SD. *P<0.01.
examined at 48 h after irradiation (4 Gy), with or without pyrotinib (0.1 µg/ml), suggesting that the combination treatment significantly increased the apoptosis of NCI-N87 cells, but not SKBR3 cells, when compared with the irradiation alone group (Fig. 4C). Treatment with pyrotinib or irradiation alone induced a significant increase of apoptosis in SKBR3 cells in comparison with control group. Additionally, irradiation alone demonstrated a significant increase of apoptosis in NCI-N87 cells. However, pyrotinib alone failed to induce any significant changes of apoptosis in NCI-N87 cells.

The effects of pyrotinib and irradiation alone, or combination on cell cycle progression were examined using flow cytometry (Fig. 4D). Following irradiation, G2/M phase cells were increased in the cell cycle distribution of both two cell lines, compared with the control group. Moreover, pyrotinib induced in G2/M arrest after irradiation only in NCI-N87 cells. In SKBR3 cells, pyrotinib failed to significantly affect irradiation-induced G2/M arrest.

These findings indicated that pyrotinib promotes apoptosis of and G2/M arrest induced by irradiation, and this may be the additional mechanism via which pyrotinib could overcome resistance to irradiation in HER2-overexpressing GC and BC.

Pyrotinib inhibits DNA double-strand break (DSB) repair in irradiated HER2-overexpressing GC and BC cells. To further determine the other potential mechanisms of pyrotinib-induced radiosensitivity, the effect of this agent on DSBs repair was investigated. The expression of γ-H2AX, a marker of DSBs, was assessed in irradiated NCI-N87 and SKBR3 cells, with or without pretreatment of pyrotinib. Pyrotinib co-treatment markedly enhanced the expression of γ-H2AX compared with irradiated cells 1 h after irradiation (Fig. 5A and B).
Additionally, γ-H2AX expression was present for a longer period in NCI-N87 cells compared with in SKBR3 cells. As DSBs repair is a well-established cause of radioresistance (21), these findings suggested that the inhibition of DSBs repair may be a common mechanism via which pyrotinib sensitizes HER2-overexpressing GC and BC cells to irradiation.

Pyrotinib enhances the cytotoxicity of docetaxel in HER2-overexpressing GC and BC cells. To investigate the possible antitumor effects of pyrotinib in combination with docetaxel, fluorouracil, cisplatin or epirubicin, NCI-N87 and SKBR3 cells were exposed to varying concentrations of each agent alone, with or without pyrotinib (0.1 µg/ml) for 48 h. The combination experiments demonstrated that pyrotinib significantly increased the cytotoxicity of docetaxel and cisplatin in NCI-N87 cells (Fig. 6). While in SKBR3 cells, pyrotinib significantly enhanced the cytotoxicity of docetaxel and fluorouracil. It was found that pyrotinib did not increase the cytotoxicity of epirubicin in both cell lines. Overall, the data indicated that pyrotinib sensitized both NCI-N87 and SKBR3 cells to docetaxel.

Discussion

The present study demonstrated that pyrotinib enhanced the radiosensitivity of HER2-overexpressing GC and BC cells, both in vitro and in vivo. It was suggested that the major mechanism involved in this process was pyrotinib-induced inhibition of HER2 nuclear transport. Additionally, the present findings indicated that pyrotinib was associated with increased DNA damage induced by irradiation. A potent capacity of pyrotinib to augment the cytotoxicity of docetaxel was observed in both GC and BC cell lines. These data could provide a strategy to improve the response to RT, as well as to select the most efficient drug combination in individual patients.

The present results suggested that inhibition of irradiation-induced HER2 nuclear transport was a crucial mechanism for pyrotinib-enhanced radiosensitivity. Although the mechanism of HER2-induced radioresistance remains unknown, increasing evidence, including data from the current study, reveal the presence and function of HER2 in the nucleus (13-20,22). Nuclear HER2 positivity has been identified as an independent prognostic factor in patients with BC with membrane HER2-upregulation (23). Reportedly, irradiation can result in HER2 nuclear transport, which may contribute to the radiosensitivity of cancer types with high HER2 expression (14). In the present study, nuclear HER2 was decreased with pyrotinib treatment in irradiated cells.

The response to irradiation is recognized to be driven by the repair efficacy of irradiation-induced DNA damage, in which DSBs serve a major role (21). Since EGFR can inhibit DNA damage (24), the present study investigated whether pyrotinib suppressed DSB repair in response to irradiation by detecting γ-H2AX expression. H2AX is rapidly phosphorylated at the site of DSBs and acts as a damage signaling protein forming nuclear foci visible using immunofluorescence (21). Compared with cells treated with irradiation alone, pyrotinib significantly markedly γ-H2AX expression in NCI-N87 and SKBR3 cells after irradiation, suggesting a failure of DSBs repair. The present findings suggested pyrotinib was associated with increased DNA damage induced by irradiation.

Cancer cell exposure to irradiation results in the activation of the HER family, subsequently stimulating downstream
signaling pathways that regulate cellular processes, including proliferation, apoptosis and cell cycle distribution (25,26). The key mechanism of irradiation-induced cell death is apoptosis (25). In the present study, pyrotinib increased irradiation-induced apoptosis only in NCI-N87 cells. The cell cycle distribution was also analyzed. Compared with irradiation alone, the combination of irradiation and pyrotinib substantially enhanced G2/M arrest in NCI-N87 cells. The enhanced radiosensitivity identified in NCI-N87 cells may be attributed to increased apoptosis and G2/M arrest, while this was not observed in SKBR3 cells. Genetic heterogeneity between the two cell lines may explain these inconsistent results. However, other factors besides HER2 may participate in the irradiation-induced apoptosis and cell cycle redistribution in BC.

Trastuzumab combined with RT has been widely used in the adjuvant therapy of BC (12,27,28). The toxicities of RT with concurrent trastuzumab are deemed acceptable and the outcomes were favorable (27,28). A clinical trial provided relevant evidence demonstrating the radiosensitizing effect of trastuzumab in HER2-overexpressing BC (29). However, tumors expressing HER2 may exhibit autocrine stimulation of EGFR/HER1 via expression of one of its numerous ligands including EGF, amphiregulin (AR) and TGF-α (30). Therefore, this type of cooperation may result in the activation of additional intracellular pathways, contributing to tumor progression. In this respect, pyrotinib, an irreversible dual (EGFR/HER2) TKI, has displayed high potency in HER2-dependent cancer cells in vitro and in vivo (20).

Figure 6. Effects of pyrotinib in combination with docetaxel, fluorouracil, cisplatin or epirubicin in NCI-N87 and SKBR3 cells. Cells were cultured with various concentrations of chemicals alone or combined with pyrotinib (0.1 µg/ml) for 48 h. Cell viability was detected using the Cell Counting Kit-8 assay. Data are presented as the mean ± SD. **P<0.01.
signal pathway, instead of the MEK/MAPK-mediated signal transduction (11). Consistently, the present study demonstrated that pyrotinib combined with irradiation markedly suppressed p-Akt expression in SKBR3 cells. By contrast, in NCI-N87 cells, pyrotinib did not notably inhibit the PI3K/Akt or MEK/MAPK pathways. Thus, the distinct mechanisms of action and chemical structures of pyrotinib and trastuzumab may induce diverse signal transduction pathways in cancer types.

The current findings support the feasibility of RT combined with pyrotinib in patients with HER2-overexpressing GC and BC. Indeed, neoadjuvant RT may achieve clinical tumor downsizing and increase the possibility of R0 resection in patients with local advanced GC (33-36). Thus, neoadjuvant RT could be developed as a promising standard treatment for patients with potentially resectable local advanced GC. Since INT-0116 and ARTIST studies were published, postoperative RT has also been utilized in patients with GC (37,38). R1 surgical resection is an indication for postoperative RT. RT is considered as a crucial component of BC therapy and has been recommended in various guidelines (e.g. National Comprehensive Cancer Network; American Society of Clinical Oncology; European Society for Medical Oncology; Chinese Society of Clinical Oncology) for >20 years (39-41). In patients harboring regional lymph node metastasis or residual tumor tissue in the chest wall after surgery or systemic therapy, RT can not only decrease the local recurrence rate but also prolong survival time (42,43).

In the present study, the use of sub-cytotoxic concentration of pyrotinib could increase the cytotoxicity induced by irradiation and the radiosensitivity of tumor tissues, without enhancing the irradiation dose and side effects of RT. Before actual clinical applications, further research is required to assess the feasibility, benefits and adverse reactions of using pyrotinib as a radiosensitizer. The clinical applications of pyrotinib are currently under investigation. For instance, studies into the tolerability, safety and pharmacokinetic properties in humans have been completed (20). The first study (clinical trial no. NCT01937689) of pyrotinib in patients with HER2-positive metastatic BC who previously received treatment with trastuzumab, or were trastuzumab naïve, reported that the median progression-free survival was 35.4 and 59.7 weeks in the 320 and 400 mg dose cohorts, respectively (44). Further investigations of pyrotinib in patients with HER2-positive metastatic BC are ongoing to collect data relating to its safety and efficacy. In addition, the present results suggested that pyrotinib enhanced the cytotoxicity of docetaxel in NCI-N87 and SKBR3 cells, which may provide a novel treatment strategy for HER2-overexpressing GC and BC.

Several limitations of the present study should be mentioned. First, as few GC cell lines overexpress HER2, except NCI-N87, confirmation of HER2 expression in additional GC cell lines would be valuable. Second, the effect of RT in combination with pyrotinib and docetaxel requires further investigation.

In conclusion, to the best of our knowledge, the present findings are the first to suggest that pyrotinib sensitized HER2-overexpressing GC and BC cells to irradiation by inhibiting HER2 nuclear transport. It was identified that DNA damage appeared to serve a crucial role in pyrotinib-induced sensitization of cancer cells to irradiation (Fig. 7). The cytotoxicity of docetaxel was enhanced by pyrotinib in HER2-overexpressing GC and BC cells. However, additional clinical investigations and associated translational studies are required to further understand the current findings.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

TH wrote the original manuscript and analyzed the data. TH, XL, BW, PP, YD and GH performed the experiments and partial data analysis. YD and GH revised the original manuscript. HQ and XY designed and revised the research for critical intellectual content. Each author made a significant scientific contribution to the present research and was familiar with the primary data. All listed authors have read the complete manuscript and approved the submission and publication of the paper.

Ethics approval and consent to participate

Experiments in the present study were executed according to the guidelines of, and were approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.
Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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