RON confers lapatinib resistance in HER2-positive breast cancer cells

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ABSTRACT

Lapatinib-resistance is a major problem for HER2-positive breast cancer treatment. SK-BR-3-LR, a lapatinib-resistant cell clone, was established from HER2-positive SK-BR-3 breast cancer cells following chronic exposure to lapatinib. The PI3K/AKT signaling pathway was demonstrated to be resistant to HER2 inhibition in SK-BR-3-LR cells. However, both small-molecular Recepteur d’Origine Nantais (RON) inhibitors and RON-targeted small interfering RNA (siRNA) effectively restored lapatinib sensitivity in these cells by inhibiting PI3K/AKT activation. Our results demonstrate for the first time the important role of RON in mediating lapatinib resistance and suggest that RON-targeted therapy may become a novel, promising therapeutic strategy after the failure of lapatinib treatment in patients with HER2-positive breast cancer.

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1. Introduction

Breast cancer is the second most frequently diagnosed malignancy worldwide, and the prognosis of patients with this disease is poor [1]. HER2 is a member of the ErbB receptor tyrosine kinase protein family, and HER2 overexpression in human breast cancers is associated with aggressive tumor growth and poor clinical prognosis [2]. Lapatinib, a reversible, ATP-competitive dual kinase inhibitor that targets EGFR and HER2, is approved for the treatment of patients with advanced or metastatic breast cancers that overexpress HER2 and for those who have received prior therapy, including anthracycline, a taxane, and trastuzumab [3]. However, most HER2-positive breast cancer patients treated with lapatinib eventually develop drug resistance.

Several mechanisms, including PTEN loss, PI3CA mutation and c-SRC overexpression, are found to mediate the resistance to HER2-targeted agents, including the humanized monoclonal anti-HER2 antibody trastuzumab and the small molecular HER2 inhibitor lapatinib [4–8]. Additionally, overexpression of AXL, a receptor tyrosine kinase, has also been reported to play an important role in lapatinib resistance through eliciting PI3K/AKT signaling activation [9].

The Recepteur d’Origine Nantais (RON)/MSP receptor (MSPR) tyrosine kinase is a member of the MET proto-oncogene family. Ligand-binding leads to RON/MSPR autophosphorylation and the activation of signaling components, including PI3K/AKT, focal adhesion kinase, and ERK1/2, which are involved in cell proliferation, tubular morphogenesis, cell motility, migration, and invasion [10–12]. RON has been recognized as an oncogene that is involved in human epithelial tumor development and growth [13] and is reported to be upregulated in various human cancers, including breast, lung, stomach, colon, pancreatic and prostate cancers [14]. In contrast to low expression in normal breast epithelium, RON overexpression has been detected in 50% of primary breast carcinomas [15]. Moreover, aberrant RON expression in breast cancer patients is associated with a worsened clinical outcome [16]; thus, RON is recognized as a molecular therapy target.

In addition to MSP-binding activation, multiple RON variants with various deletions or truncations in the extracellular or intracellular regions have been identified [17], and all of these variants are constitutively activated and contribute to the invasive phenotype and malignant progression.

In this study, we established a lapatinib-resistant cell monoclonal, SK-BR-3-LR, from HER2 positive SK-BR-3 human breast cancer cells. The PI3K/AKT signaling pathway was found to be resistant to HER2 inhibition in SK-BR-3-LR cells; however, both small-molecular RON inhibitors and RON-targeted small interfering RNA (siRNA) effectively restored the sensitivity of these cells to lapatinib through inhibiting PI3K/AKT activation. Our data provide evidence that RON confers lapatinib resistance, and RON may be a new target for overcoming lapatinib treatment resistance in HER2-positive breast cancer.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies against HER2, phospho-HER2 (Tyr1221/1222), EGFR, phospho-EGFR (Tyr1173), c-MET, phospho-c-MET (Tyr1234/1235), AKT, phospho-AKT (Ser473), ERK1/2, phospho-ERK1/2 (Thr202/Tyr204), caspase-3, caspase-8, poly-(ADP-ribose) polymerase (PARP) were purchased from Cell Signaling (Beverly, MA, USA). The RON C-terminal antibody (C-20) was obtained from Santa Cruz Biotechnology (San-
ta Cruz, CA), the anti-phosphotyrosine antibody (clone 4G10) was obtained from Millipore Corporation (Billerica, MA, USA), the human RON N-terminal antibody was purchased from Epitomics, Inc., and the β-tubulin antibody was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Lapatinib, dasatinib, sunitinib, XL880, PHA 665752, JNJ 38877605, MGCD-265, GDC-0941, HKT-272 and PD 0325901 were obtained from Selleckchem (Houston, TX, http://www.selleckchem.com); c-MET/RON dual kinase inhibitor, alternately named RON inhibitor I, was purchased from the Millipore Corporation (Billerica, MA, USA). All compounds were prepared in DMSO at a concentration of 10 mM and stored at -20 °C. Herceptin was purchased from F. Hoffmann-La Roche Ltd. (manufactured by Genentech Inc., South San Francisco, California, USA). rhHGF and MSP were purchased from R&D Systems, Inc.

2.2. Cell culture, treatment, and transient transfection

The human SK-BR-3 cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were plated in six-well plates and cultured in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum at 37 °C in a humidified 5% carbon dioxide atmosphere. Prior to drug treatment, the cells were grown until they reached 50–60% confluence. For siRNA studies, the cells were transfected with siRNA targeting c-MET, RON or a negative control siRNA (Santa Cruz, CA) at a final concentration of 30 nM using the lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. After transfection for 48 h, the cells were harvested for protein extraction and additional analyses. All experiments were performed in triplicate, and representative results were reported.

2.3. Cell proliferation assay

Cell growth inhibition was determined using a sulforhodamine B assay, as described previously [18]. Briefly, approximately 24 h after plating, the cells were exposed to compounds with 3.16-fold serial dilutions alone or a combination of the two agents, as indicated. The cells were incubated with the compounds in culture medium containing 10% fetal bovine serum for 72 h. At least three independent experiments were performed, and the results represent the mean ± SD.

2.4. Immunoprecipitation and Western blotting

Western blotting was performed as described previously [18]. For immunoprecipitation, the treated cells were lysed in lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 1 mM Na3VO4, 10 mM Na4P2O7, 100 mM NaF, 10 μg/ml leupeptine, 10 U/ml aprotinin and 1 mM PMSF). Cell lysates were clarified by microcentrifugation, and the supernatant was incubated with appropriate antibodies overnight at 4 °C and incubated with protein G agarose beads (GE Healthcare Life Sciences) for another 3 h. The beads were microcentrifuged, washed and resuspended with 3 × SDS buffer. The immunoprecipitates were resolved using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrotransferred onto PVDF membranes, and the proteins were detected by immunoblotting using the Western blot Imaging System (Clinx Science Instruments Co., Ltd.).

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 (Mean ± SD, nM)</th>
<th>Growth inhibition (%) (1 μg/ml Herceptin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lapatinib</td>
<td>HKI-272</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>74.8 ± 18.4</td>
<td>8.0 ± 0.8</td>
</tr>
<tr>
<td>SK-BR-3-LR</td>
<td>&gt;10,000</td>
<td>214.1 ± 11.2</td>
</tr>
</tbody>
</table>

| SK-BR-3 and SK-BR-3-LR cells were treated with different concentrations of agents for 72 h, and IC50 (concentration of 50% growth inhibition) were determined by the sulforhodamine B assay. Three independent experiments were performed, and the results were presented the mean ± SD.

A

B

C

D

Fig. 1. Aberrantly activated PI3K/AKT pathway mediates lapatinib resistance in SK-BR-3-LR cells. (A and B) After drug treatment, phosphorylation of HER2, EGFR, AKT, and ERK1/2 was determined by Western blotting using specific antibodies. (C) Growth inhibitory effects of GDC-0941 on SK-BR-3 and SK-BR-3-LR cells. (D) Growth inhibitory effects of lapatinib alone or in combination with 0.3 μM GDC-0941 or 1 μM PD 0325901 on SK-BR-3-LR cells. Cell growth was determined using the sulforhodamine B assay.
2.5. Reverse transcription PCR

Total RNA was extracted from the cells using the Trizol reagent system (Sangon, Shanghai, China) and quantitated (SPECTRA max190; Molecular Devices). Complementary DNA was synthesized using the PrimeScript® Reverse Transcription reagent Kit (Takara, Dalian, China) according to the manufacturer’s instructions. PCRs were performed using the PrimeSTAR® HS DNA Polymerase (Takara) on the T100™ Thermal Cycler PCR system (BIO-RAD). These PCRs were performed using primers against human RON-delta-165 (forward: 5’-CCAACCTGCTACATCAACT-3’ and reverse: 5’-ATGCCCTCAGGCTTCAGT-3’) and RON-delta-160 (forward: 5’-CACAATGGGTCGCTATGAT-3’ and reverse: 5’-AAGGCTCCACGTCACACT-3’). These primers were purchased from Sangon (Shanghai, China).

2.6. Data analysis

Data were analyzed using the GraphPad Prism software. Nonlinear regression analyses were performed to generate dose–response curves and to calculate IC50 values. The mean ± SD was calculated automatically using this software. A paired, two-tailed Student’s t-test was used to determine the significance where indicated.

3. Results

3.1. Establishment of lapatinib-resistant SK-BR-3-LR breast cancer cells

The SK-BR-3 cells were grown initially in medium containing 0.05 μM lapatinib, and the concentration was gradually increased to 2 μM over the subsequent 12 months to establish lapatinib-resistant cell lines (SK-BR-3-LR). Cell growth assays were performed for the SK-BR-3 and SK-BR-3-LR cells with various concentrations of lapatinib. As shown in Table 1, the IC50 (concentration of 50% growth inhibition) value of lapatinib in SK-BR-3-LR (>10 μM) was over 100-fold higher than that in SK-BR-3 (0.06 μM), indicating a significant resistance to lapatinib in SK-BR-3-LR cells. Furthermore, SK-BR-3-LR cells were also resistant to other HER2-targeted agents, such as HKI-272 and Herceptin, but were still sensitive to the microtubule-disrupting agent vinorelbine and the heat shock protein 90 inhibitor geldanamycin (Table 1).

![Fig. 2. c-MET/RON inhibitors restore sensitivity to lapatinib in SK-BR-3-LR cells.](image-url)
3.2. PI3K/AKT signaling pathway is insensitive to HER2 inhibition in SK-BR-3-LR cells

To examine the mechanism of lapatinib resistance in SK-BR-3-LR cells, we first determined the phosphorylation status of HER family kinases and downstream effectors of the PI3K/AKT and ERK1/2 pathways in SK-BR-3 and SK-BR-3-LR cells. Lapatinib inhibited HER2 and EGFR phosphorylation in both cell lines; however, it significantly decreased phosphorylation levels of AKT (S473) and ERK1/2 only in SK-BR-3 cells but not in SK-BR-3-LR cells (Fig. 1A). Additionally, GDC-0941, a specific PI3K inhibitor, significantly decreased AKT phosphorylation in both SK-BR-3 and SK-BR-3-LR cell lines (Fig. 1B) and inhibited the proliferation of both cell lines with the same potency (Fig. 1C). Moreover, GDC-0941 synergistically enhanced the inhibitory effect of lapatinib in SK-BR-3-LR cells with the IC₅₀ > 10 μM for lapatinib alone decreased to 0.55 μM for the combination (Fig. 1D). In contrast, the MEK inhibitor PD0325901, which inhibited ERK1/2 activation in both the SK-BR-3 and SK-BR-3-LR cells, did not show any growth inhibitory effect in these cells (data not shown) and exhibited no synergistic effects with lapatinib in SK-BR-3-LR cells (Fig. 1D). Collectively, these results indicate that the activated PI3K/AKT signaling pathway confers lapatinib resistance in SK-BR-3-LR cells and imply the presence of a novel event responsible for PI3K/AKT activation.

3.3. RON inhibitor I inhibits PI3K/AKT signaling and restores lapatinib sensitivity in SK-BR-3-LR cells

To determine which upstream event in SK-BR-3-LR cells was responsible for PI3K/AKT activation, these cells were treated with inhibitors of various kinases plus lapatinib to determine which of the combinations had a synergistic growth-inhibitory effect. As shown in Fig. 2A, crizotinib, a c-MET, RON and ALK kinase inhibitor [19], MGCD-265, a multi-targeted kinase inhibitor targeting c-MET, VEGFR1/2/3 and RON [20] and XL880, a multi-targeted kinase inhibitor of c-MET, KDR, FLT3, PDGFR and RON [21], could significantly restore lapatinib sensitivity in SK-BR-3-LR cells when combined with lapatinib at a concentration of 0.1 μM. In contrast, other kinase inhibitors, including the specific ALK inhibitor TAE-684 [22], the VEGFR/PDGFR/c-Kit/FLT3 inhibitor sunitinib [23], and the BCR-ABL/SRC inhibitor dasatinib [24], did not synergistically enhance the growth inhibition effect of lapatinib in SK-BR-3-LR cells (Fig. 2A). Consistent with the cell proliferation data, the Western blot results demonstrated that only crizotinib, MGCD-265 and XL880 (Fig. 2B), but not sunitinib, TAE-684, and dasatinib (Supplementary Fig. 1), significantly inhibited AKT and ERK1/2 phosphorylation in SK-BR-3-LR cells when combined with lapatinib. Taken together, these results suggest that crizotinib, MGCD-265 and XL880 may inhibit one key kinase that is responsible for lapatinib-insensitive AKT phosphorylation. Previous reports demonstrated that c-MET and MSPR/RON were the common targets of crizotinib, MGCD-265 and XL880 [20,25,26]: thus, we examined whether RON inhibitor I, a selective dual c-MET and RON inhibitor [27], was able to mimic the same effects as crizotinib, MGCD-265 and XL880. As expected, RON inhibitor I plus lapatinib significantly decreased AKT and ERK1/2 phosphorylation in the SK-BR-3-LR cells (Fig. 2C). Furthermore, when used in combination with lapatinib and Herceptin in SK-BR-3-LR cells, RON inhibitor I potently restored the sensitivity of these cells (Fig. 2D and E). Moreover, this drug co-treatment resulted in SK-BR-3-LR cell apoptosis as demonstrated by PARP and caspase cleavage, whereas no significant apoptosis was observed when lapatinib was used alone (Fig. 2F). Thus, the results above indicate that c-MET or RON kinase may play an essential role in lapatinib-resistance in SK-BR-3-LR cells.

Fig. 3. c-MET is not responsible for the lapatinib-resistance in SK-BR-3-LR cells. (A) SK-BR-3-LR cells were treated with 0.1 μM PHA 665752 or JNJ 38877605 for 1.5 h, and then stimulated with 100 ng/ml rhHGF for 10 min. (B) Cells were treated as indicated. The phosphorylation of HER2, AKT and ERK1/2 was determined by Western blotting. (C) SK-BR-3-LR cells were treated with various concentrations of lapatinib alone or in combination with 0.1 μM PHA 665752, and cell growth was evaluated using sulforhodamine B assays. (D) SK-BR-3-LR cells were transfected with negative control siRNA (lane 1, lane 2) and c-MET-targeted siRNA (lane 3, lane 4). Cells were treated, 48 h later, with 0.1 μM lapatinib for 1.5 h, and then total cell lysates were prepared and analyzed by Western blotting.
Considering that c-MET has been involved in drug resistance to several tyrosine kinase inhibitors [28–30], we investigated whether c-MET dominated the SK-BR-3-LR cells' resistance to lapatinib. No difference in c-MET expression was observed, and c-MET phosphorylation could not be detected in either parental or resistant cells (data not shown), indicating that c-MET activity was not likely responsible for lapatinib resistance in SK-BR-3-LR cells. Moreover, PHA 665752 and JNJ 38877605, two highly selective c-MET inhibitors that did not demonstrate RON inhibition at 1 μM [31], potently inhibited HGF-induced c-MET phosphorylation (Fig. 3A) but did not synergistically enhance the downstream signaling inhibition or proliferation inhibition of lapatinib in SK-BR-3-LR cells (Fig. 3B and C). Furthermore, c-MET protein knockdown using c-MET-targeted small-interfering RNA did not decrease AKT and ERK1/2 phosphorylation, either alone or in combination with lapatinib (Fig. 3D). Collectively, these results indicate that c-MET is not involved in lapatinib resistance in SK-BR-3-LR cells.

3.4. RON/PI3K/AKT activation confers lapatinib resistance

Because c-MET was excluded, we proposed that RON might be the key kinase in mediating lapatinib resistance in SK-BR-3-LR cells. To test this hypothesis, RON-targeted small interfering RNA (siRNA) was used to transfsect SK-BR-3-LR cells, which resulted in significantly decreased RON protein expression and reduced phosphorylated AKT levels (Fig. 4A) and increased lapatinib sensitivity. Moreover, the cells treated with control siRNA did not demonstrate these results (Fig. 4B), thus supporting our hypothesis that RON contributes to lapatinib resistance in SK-BR-3-LR cells.

RON short variants have been reported to be constitutively activated and to stimulate downstream signaling pathways [17,27]. A phosphorylated RON short variant was observed in SK-BR-3-LR cells, but not in SK-BR-3 cells, despite the identical pro-RON and RON α-chain (α-RON) expression levels in both cells (Fig. 4C). Additionally, the phosphorylated RON short variant and downstream AKT and ERK1/2 phosphorylation could be inhibited by RON inhibitor 1 in a dose-dependent manner with IC50 of 0.05 μM (Fig. 4D), which was consistent with a previous report [27]. Thus, these results indicate that RON activation mediates downstream PI3K/AKT pathway activation and results in lapatinib resistance in SK-BR-3-LR cells.

To further support the evidence for the role of RON in acquired lapatinib resistance, the macrophage-stimulating protein (MSP) was used in combination with lapatinib in parental SK-BR-3 cells. In addition to inducing RON phosphorylation and increasing the phosphorylation of downstream AKT and ERK1/2, MSP was able to partially rescue lapatinib’s inhibition of AKT and ERK1/2 (Fig. 5A). Furthermore, the cell growth inhibition of lapatinib was

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**Fig. 4.** Aberrantly activated RON confers lapatinib resistance in SK-BR-3-LR cells. (A) SK-BR-3-LR cells were transfected with negative control siRNA (lane 1 and lane 2) or RON-targeted siRNA (lane 3 and lane 4). Cells were treated, 48 h later, with 0.1 μM lapatinib for 1.5 h, and then total cell lysates were prepared and analyzed by Western blotting. (B) After SK-BR-3-LR cells were transfected with RON-targeted siRNA or negative control siRNA, cell growth was determined by sulforhodamine B assay. (C and D) α-RON and pro-RON were detected by a RON N-terminal antibody. β-RON was immunoprecipitated using an antibody against the RON C-terminus, and then its phosphorylation was detected using a 4G10 antibody. Arrows, positions of the molecular weight marker. α-RON: RON α-chain; β-RON: RON β-chain.
also partially rescued by MSP (Fig. 5B). These results provide further evidence that RON confers lapatinib resistance to SK-BR-3 cells.

4. Discussion

Drug resistance inevitably occurs following long-term, HER2-directed treatment in patients with breast cancer; thus, understanding the mechanism underlying such drug resistance and finding strategies to overcome this critical problem is of clinical importance. In this report, a lapatinib-resistant clone SK-BR-3-LR was established from HER2-positive SK-BR-3 human breast cancer cells following the chronic exposure to gradually increasing amounts of lapatinib. Although several mechanisms of lapatinib resistance have been reported, we demonstrate for the first time that the RON tyrosine kinase confers resistance to lapatinib in HER2-positive breast cancer cells. We believe that our findings will facilitate the development of rational strategies to overcome lapatinib resistance in the clinic.

RON/MSPR is a membrane-bound receptor tyrosine kinase closely related to c-MET and is involved in tumorigenesis. RON overexpression has been detected in 50% of primary breast carcinomas, and RON was previously reported to confer tamoxifen resistance on breast cancer cell lines [32]. Recently, a RON short variant was demonstrated in acute myeloid leukemia and sensitized leukemic cells to c-MET/RON inhibitors [33]. In our current study, we show a definitive link between RON activation and lapatinib resistance in SK-BR-3-LR cells based on the following evidence: a, the activated RON short variant was detected only in SK-BR-3-LR cells; b, both RON inhibitors and RON-targeted siRNA restored the sensitivity to lapatinib by inhibiting PI3K/AKT signaling in SK-BR-3-LR cells; and c, the RON agonist MSP partially rescued the lapatinib mediated inhibition of SK-BR-3 cell proliferation.

MSP-binding-induced activation and RON short variant autoactivation are two mechanisms of RON activation [11]. In this study, no phosphorylation of full-length RON was detected in the parental and resistant SK-BR-3 cells, whereas the phosphorylated RON short variant was detected in SK-BR-3-LR cells, but not in SK-BR-3 cells, indicating that autoactivation of RON short variants contribute to RON and downstream PI3K/AKT activation in SK-BR-3-LR cells. RON short variants have been reported to possibly be generated from RON mRNA splice variants, truncations in the extracellular region, or hypermethylation of the distal CpG island in the RON proximal promoter [17,34,35]. There are about six RON variants have been reported [17], Except for RONA170, which generated by pre-mRNA splicing that eliminates exon 19 coding the kinase domain of the RON [18], all changes in the RON sequence lead to the constitutive activation. RONA165 produced by a spliced mRNA transcript with an in-frame deletion in exon 11. RONA160 derived from a splicing mRNA transcript that has an in-frame deletion of 109 amino acids coded by exons 5 and 6 in the RON β-chain extracellular sequences. The deletion of exon 11 prevents the maturation of RONA155 into the α/β two-chain form and the protein is retained in the cytoplasm [34]. RONA110 is a proteolytically truncated RON variant [11]. And RONA55 also named short form RON is generated from alternative transcription at Met913 in the RON gene [36]. To investigate the mechanism underlying RON variant production, exons 5, 6 and 11 of RON cDNA were amplified to detect whether RON Δ155, Δ160 or Δ165 were generated from RON mRNA splice variants in SK-BR-3-LR and SK-BR-3 cells. No difference in PCR production from these two cell lines was observed (data not shown), indicating that RON short variant does not result from RON mRNA splice variants in SK-BR-3-LR cells. Thus, the mechanism of this RON variant production requires further investigation.

c-MET amplification has been frequently reported to be involved in cellular drug resistance to several tyrosine kinase inhibitors including gefitinib, lapatinib and sunitinib [28-30]. Our data showed that crizotinib, MGCD-265, XL880 and RON inhibitor I, which are potent c-MET inhibitors, significantly restored lapatinib sensitivity in SK-BR-3-LR cells; thus, it is theoretically likely that c-MET mediates lapatinib resistance in SK-BR-3-LR cells. However, c-MET siRNA failed to mimic the effect of these inhibitors, and RON inhibition was enough to reverse lapatinib resistance, and clearly excludes the possibility of c-MET involvement.

RON confers lapatinib resistance in SK-BR-3-LR cells, however, RON inhibition alone is not sufficient to inhibit the proliferation of these resistant cells because only the combination of RON inhibitor I and lapatinib was able to completely block AKT phosphorylation (Fig. 2C) and induce more apoptosis (Fig. 2F) of SK-BR-3-LR cells. We believe that dual inhibition of RON and HER2 is required for overcoming lapatinib resistance.

Fig. 5. MSP, the ligand for RON, is able to partially rescue the effect of lapatinib in SK-BR-3 cells. (A) Cells were treated with 0.1 μM lapatinib or 0.1 μM lapatinib plus 0.1 μM RON inhibitor I for 1.5 h, and then stimulated with 30 ng/ml MSP for another 10 min. Total cell lysates were prepared and analyzed by Western blotting. (B) SK-BR-3 cells were treated with 0.1 μM lapatinib alone or 0.1 μM lapatinib plus 30 ng/ml MSP for 72 h. Cell growth was determined using sulforhodamine B assays. Columns, means; bars, SEMs (n = 3; *P < 0.05 vs. lapatinib).
In summary, we demonstrate a novel mechanism in which RON kinase mediates lapatinib resistance in HER-2-positive breast cancer cells. Our findings provide evidence that RON-targeted therapy may be a novel, promising treatment strategy after lapatinib failure in patients with HER2-positive breast cancer.

5. Conflicts of interest

There are no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2013.06.022.

References

