MET and ERBB2 Are Coexpressed in ERBB2\(^{+}\) Breast Cancer and Contribute to Innate Resistance

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**Abstract**

Breast cancer displays significant intratumoral heterogeneity, which has been shown to have a substantial impact on both innate and acquired resistance to tyrosine kinase inhibitors. The heterogeneous expression of multiple receptor tyrosine kinases (RTK) in cancers supports tumor signaling robustness and plays a significant role in resistance to targeted inhibition. Recent studies have revealed interactions between the MET receptor and the ERBB receptor family in the therapeutic resistance of several cancers. In this study, the relationship between MET expression/activity and the expression/activity of the ERBB receptor family in human breast cancer was interrogated. Importantly, a significant percentage of ERBB2\(^{+}\) tumors coexpressing MET and ERBB2 were observed and displayed significant heterogeneity with subpopulations of cells that are MET\(^{+}\)/ERBB2\(^{-}\), MET\(^{-}\)/ERBB2\(^{+}\), and MET\(^{-}\)/ERBB2\(^{-}\). In a MET\(^{-}\)/ERBB2\(^{+}\) breast cancer cell line, MET depletion resulted in increased ERBB2 activation, and conversely, ERBB2 depletion resulted in increased MET activation. Neither EGFR nor ERBB3 compensated for MET or ERBB2 knockdown. The loss of either MET or ERBB2 led to a decrease in PI3K/AKT signaling and increased dependency on MAPK. These data show that a subset of ERBB2\(^{+}\) breast cancers express MET and contain MET\(^{-}\)/ERBB2\(^{+}\) subpopulations. Moreover, analysis of RTK activation during ERBB2 knockdown indicated that MET signaling is a compensatory pathway of resistance.

**Implications:** ERBB2\(^{+}\) breast cancers with MET\(^{-}\)/ERBB2\(^{+}\) subpopulations may have an innate resistance to ERBB2 inhibition and may benefit from combined MET and ERBB2 inhibition. *Mol Cancer Res; 11(9); 1112–21. ©2013 AACR.

**Introduction**

Breast cancers display a remarkable phenotypic diversity that is exploited to promote both tumor progression and therapeutic resistance. Advances in our understanding of tumor heterogeneity have been used to develop effective biomarkers and therapeutic strategies for several breast cancer subtypes. In particular, therapies targeting the estrogen receptor (ER) and ERBB2 signaling have had a significant impact on breast cancer mortality. The pioneering concept of identifying patient cohorts that overexpress ERBB2 and treating them with the targeted inhibitor trastuzumab (Herceptin) proved to be an effective strategy and is now the standard course of treatment for ERBB2\(^{+}\) patients (1, 2). In spite of this promising start, targeted inhibition of tyrosine kinases has had limited success in the clinic. Both innate and acquired resistance are significant clinical issues for tyrosine kinase inhibitors (TKI), including trastuzumab. To develop effective treatment strategies, it is necessary for us to understand how tumor heterogeneity is driving resistance to targeted therapies.

Recent studies in several types of cancer have highlighted the significance of intratumoral heterogeneity for both innate and acquired resistance (3–5). The heterogeneous expression of multiple receptor tyrosine kinases (RTK) throughout the tumor is a principal mechanism of resistance. Several studies have shown that targeted inhibition of one RTK is ineffective due to upregulation or ligand stimulation of other RTKs (6, 7). Engagement of these compensatory kinases maintains signaling robustness through crucial cell-survival effectors, principally phosphoinositide 3-kinase PI(3)K and mitogen-activated protein kinase (MAPK ref. 6, 8). Tumor plasticity is maintained by the heterogeneous expression of RTKs and the robustness that the overlapping signaling networks...
provide. This is evident in non–small cell lung cancer (NSCLC), where resistance to EGF receptor (EGFR) inhibitors can occur through MET amplification or autocrine production of the MET ligand, hepatocyte growth factor (HGF; refs. 7, 9). Studies of other cancers have revealed that activation of compensatory RTKs is common during resistance. A recent study by Wilson and colleagues revealed the extensive redundancy in RTK signaling that exists in most cancers and showed that RTK ligands can easily drive innate resistance to TKIs (6). Therefore, a thorough understanding of the intratumoral heterogeneity is necessary for the development of effective therapeutic strategies for each patient.

For decades, the intratumoral diversity of breast cancer subtypes has been evaluated through pathologic and biomarker analysis. This information results in the diagnostic profile of tumor grade, hormonal status [estrogen receptor and progesterone receptor (PR)], and ERBB2 status. We now appreciate the extent of this intratumoral heterogeneity at the molecular level and currently classify breast cancers into 6 molecular subtypes: luminal A, luminal B, ERBB2 (HER2)+, normal-like, basal, and claudin-low. The luminal subtypes commonly depend on ER or PR signaling and have the best clinical outcome. The basal and claudin-low subtypes have the worst clinical outcome and an effective targeted therapy for these subtypes has yet to be identified. Even though trastuzumab treatment of ERBB2+ breast cancers is an effective therapy, almost half of the patients with breast cancer that overexpress ERBB2 are either non-responsive to trastuzumab or develop resistance (10–12). For clinical success to become a reality for patients with basal, claudin-low, or trastuzumab-resistant breast cancer, it is imperative that we understand the intratumoral diversity within these subtypes and the compensatory mechanisms by which resistance arises.

The oncogene MET encodes a RTK that is involved in the progression and metastasis of most solid human cancers (13). Under normal physiologic conditions, MET is activated through paracrine binding of its ligand, HGF. Under neoplastic conditions, aberrant MET signaling can occur through MET amplification, overexpression of MET and/or HGF, autocrine signaling, or mutational activation. MET is overexpressed in 20% to 30% of breast cancer cases and is a strong, independent predictor of poor clinical outcome (14–18). Previously, we discovered that mutationally activated Met is able to initiate aggressive breast carcinomas in mice, and that in humans, MET overexpression highly correlates with ER+/HER2+ and basal–like breast cancers (19, 20). These results and the efficacy of MET inhibitors against other cancers (21) suggest that MET may be an effective clinical target for aggressive breast cancer subtypes.

Recent studies have exposed interactions between MET and the ERBB receptor family in the progression and therapeutic resistance of several cancers. For example, MET amplification or activation is a mechanism of resistance to EGFR inhibition in both NSCLC and colorectal carcinomas (7, 22). Conversely, in MET-addicted gastric carcinomas, activation of the ERBB receptors EGFR and ERBB3 main-

Materials and Methods

Immunohistochemical staining and analysis

Primary breast cancer specimens were collected at Spectrum Health in 2010–2012 under a protocol approved by the Institutional Review Boards of both Spectrum Health and the Van Andel Research Institute (Grand Rapids, MI). Histopathologic diagnosis and ER, PR, and ERBB2 status were determined by a clinical pathologist at Spectrum Health (Supplementary Table S1). For MET/ERBB2 staining, heat-induced epitope retrieval with an EDTA/borate/Tris buffer (Ventana Medical Systems) was used and detection was carried out with a Ventana Discovery XT immunostainer (Ventana Medical Systems). Primary antibodies were revealed using both an UltraMap anti-Mouse Alkaline Phosphatase (red) and an UltraMap anti-Rabbit DAB (brown) detection kit (Ventana Medical Systems); hematoxylin was used as a nuclear counterstain. Primary antibodies for staining were Met-4 (24) and c-erbB-2 (Dako, A0485).

For analysis of MET and ERBB2 staining, images (3–4 optical fields per slide) were taken at ×200 magnification using a Nikon Eclipse 80i microscope (Nikon) and a CRi Nuance multispectral camera (Caliper). The images were processed by spectral unmixing with the Nuance image processing software (version 3.0.0). The supplied colocalization tool was used to determine the percentage of single- and double-positive pixels per region of interest.

Vectors and lentiviral infections

The HCC1954 cell line was obtained from American Type Culture Collection. Lentiviral-based pLKO.1 MET
and ERBB2 shRNA vectors were obtained from Sigma Aldrich. The control shRNA vector (pLKO.1 scrambled shRNA) and packaging vectors (psPAX2 and pMD2.G) were from Addgene. Briefly, to generate lentivirus, MET or ERBB2 pLKO.1 lentiviral shRNA knockdown constructs or a control-scrambled shRNA construct was transfected into HEK293T cells together with packaging plasmids (psPAX2 and pMD2.g) using Fugene HD (Roche). Filtered conditioned media was used to infect HCC1954 target cells in the presence of 8 μg/mL polybrene. Polyclonal pools of stably infected cells were selected in puromycin (1 μg/mL) containing medium for 5 days. The selected cells were serum starved for 24 hours and then were treated with heregulin (10 ng/mL; Thermo Fisher Scientific) or HGF (100 ng/mL) for 24 hours. The MET-targeting shRNA sequence used in the lentiviral construct was 5’-CCGGGCCAGCTGAAT-GATGACATTCTCGAGAATGTCATCATTCAGGCTT-GGCTTTTGT-3’ (TRCN0000040047). The ERBB2-targeting shRNA sequence used in the lentiviral construct was 5’-CGGTTGTCATATCCAGGCTTGGTACTCGAGTACAAAGCCTGGATCTGACATTTTTG-3’ (TRCN0000039878).

Western blot analysis
Whole-cell lysates were collected in a radiolimunoprecipitation assay buffer containing Complete Protease Inhibitor and PhosSTOP (Roche). Lysates (20–40 μg) were resolved on a 4–20% TGX SDS–PAGE gel (Bio-Rad) and transferred to a nitrocellulose membrane (Invitrogen). After blocking for 1 hour with 5% dry milk in TBST buffer (20 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 0.1% Tween-20), blots were probed overnight at 4°C with the following primary antibodies from Cell Signaling Technology: Met (DC12), P-MET (Y1234/Y1235; D26), P-ERBB2 (Tyr1221/1222; 6B12), P-ERBB3 (21D3), EGFR (D381), P–EGFR (Tyr1068; D7A5), AKT (#9272), P-AKT (S473; #9271), MAPK (#9102), P-MAPK (Thr202/Tyr204; #9101), and β-tubulin (#2146). ERBB2 (Ab3) antibodies were from Calbiochem and ERBB3 (C-17) antibodies were from Santa Cruz. Blots were reacted with peroxidase-conjugated antibody for 1 hour, followed by visualizing the proteins using ECL detection reagents (Amersham). Densitometric analysis of 2 independent experiments was carried out using Image J software (NIH). All signals were normalized to the loading control (β-tubulin).

³H-thymidine incorporation assay
For this assay, HCC1954 cells were infected with control shRNA, MET shRNA, or ERBB2 shRNA and puromycin-selected as described above. The cells were trypsinized and 8 × 10⁴ cells were seeded in each well of a 96-well dish. After 24 hours of serum starvation, the cells were treated with 100 ng/mL HGF or 10 ng/mL heregulin for 20 hours. The cells were then treated with 0.5 μCi of ³H-thymidine (GE Lifesciences) per well and incubated at 37°C for 4 hours. Following ³H-thymidine incorporation, the cells were washed twice with ice-cold DPBS, treated with 200 μL of ice-cold 5% TCA on ice for 20 minutes, washed once with 95% ethanol, air-dried, and lysed in 75 μL of lysis solution (0.02 N NaOH, 0.1% SDS) for 15 minutes. The cell lysates were then transferred to a scintillation reader plate (Wallac) with 200 μL of scintillation fluid (Perkin-Elmer). The incorporated ³H radioactivity was measured as cpm in the MicroBeta TriLux 1450 LSC & Luminescence Counter (Perkin-Elmer). The assays were repeated 3 times; for each assay, 6 replicates/treatment were conducted.

Results
MET and ERBB2 are coexpressed in subpopulations of cells in human breast carcinomas
In previous studies, we observed that high MET expression was associated with ERBB2+ breast cancers (19). This observation was supported by a clinical study showing that MET correlated with poor outcome independent of ERBB2 (18). To determine whether we could visualize this inverse expression pattern within a tumor, we conducted coimmunohistochemical staining of MET and ERBB2 in human breast cancer tissues. MET and ERBB2 signals and their distribution area in the tumor were calculated numerically by spectral unmixing. In tumors that were clinically defined as “triple-negative” (ER+/PR-/ERBB2-), we observed high levels of MET expression (Fig. 1A and B), which were expected given that we had previously observed increased MET in ER+/ERBB2- cases (19). In some ERBB2+ cases, we found low to no MET expression (Fig. 1C and D), but the majority of ERBB2+ tumors expressed moderate to high levels of MET (Fig. 1E and F). Coexpression of MET and ERBB2 was typically observed at the plasma membrane of tumor cells (Fig. 1E and F). The presence of both MET and ERBB2 in a tumor is supported by recent work of Wilson and colleagues, who observed MET in ERBB2+ tumors (6). However, this is the first time MET and ERBB2 coexisting has been carried out where MET and ERBB2 expression patterns were analyzed.

The intratumoral heterogeneity that is prevalent in breast cancer was clearly shown by the expression patterns of MET and ERBB2 in several breast cancers. For each slide, we measured expression in 3 to 4 optical fields, and we frequently observed distinct expression patterns within each tumor section. For example in case #34, which is an ER+/PR-/ERBB2- tumor, we observed significant heterogeneity in MET and ERBB2 expression. Two optical fields showed high MET expression (Fig. 2A and B), whereas other areas showed higher ERBB2 expression (Fig. 2C) and mixed MET and ERBB2 expression (Fig. 2D).

To determine the diversity of MET/ERBB2 expression patterns, we quantitated MET and ERBB2 expression in 30 primary breast cancers of all subtypes (Supplementary Table S1). In ERBB2+ tumors, MET expression was highest in ER tumors compared with ER- tumors (Fig. 3A). As expected, ERBB2 expression patterns were low in all of the samples clinically defined as ERBB2-+. We then examined ERBB2+ tumors and observed levels of MET expression ranging from 0% to 60% of pixels (Fig. 3B). The majority of ERBB2+ tumors expressed MET, but the percentage of MET-
MET and ERBB2 are coexpressed in human breast carcinomas. Coimmunohistochemical staining with MET and ERBB2 antibodies was carried out on human breast carcinoma sections. Multispectral imaging and spectral unmixing were carried out to identify MET (green), ERBB2 (red), and colocalized signals (yellow). A, triple-negative tumor (#16) with high MET expression and low MET/ERBB2 coexpression; B, triple-negative tumor (#4) with high MET expression and low MET/ERBB2 coexpression; C, ERBB2+ tumor (#85) with no MET expression; D, ERBB2+ tumor (#77) with no MET expression; E, ERBB2+ tumor (#6) with moderate MET expression and 10% MET/ERBB2 coexpression; F, ERBB2+ tumor (#5) with high MET expression and 45% MET/ERBB2 coexpression.

MET+/ERBB2+ cells varied significantly. For example, 52% of tumor #5 was MET+ and 45% was MET+/ERBB2+. In contrast, in tumor #54, 14% of the tumor was MET+, yet only 5% was MET+/ERBB2+. In other words, tumor #5 had a significant MET+/ERBB2+ subpopulation, whereas #42 had a considerable MET+/ERBB2− subpopulation. These...
analyses show that ERBB2 tumors are composed of subpopulations of MET/ERBB2+, MET+/ERBB2−, and MET−/ERBB2+ cells. These diverse expression patterns support the results of several studies in other cancer types that have shown intratumoral heterogeneity (3, 25) and which have significant implications for the development of optimal treatment strategies.

MET and ERBB2 compensate for each other during targeted knockdown

The coexpression of MET and ERBB2 in ERBB2+ breast cancers suggests that MET expression may contribute to their robustness. To explore whether intratumoral heterogeneity is driving innate resistance to targeted inhibition through MET–ERBB crosstalk signaling, we used the breast cancer cell line HCC1954 due to its high levels of MET and ERBB2 expression. We used lentiviral shRNA–mediated knockdown to interrogate the signaling pathways altered or maintained by tumor heterogeneity. To determine how external signals from the tumor microenvironment affect kinase signaling in these cells, we also treated the cells with either HGF or the ERBB ligand, heregulin (HRG). We note that during puromycin selection of the shRNA-infected cells a substantial loss in cell numbers was observed. MET shRNA caused cell death in 10% to 25% of cells, whereas ERBB2 shRNA induced a loss of 30% to 50% of cells. This is likely due to the addiction of these cells to amplified levels of ERBB2. Therefore, the cells remaining after selection, which were used in the following assays, are likely biased toward those with inherent plasticity and capacity for resistance.

We achieved more than 90% Met knockdown in the cells and approximately 80% ERBB2 knockdown (Fig. 4A and B). Because of extremely high ERBB2 expression, we were unable to achieve 100% knockdown. As expected, in control cells, we observed a slight increase in P–MET with HGF treatment and an increase in P–ERBB2 with heregulin treatment. In MET-KD cells, we observed an increase in P–ERBB2 in all 3 conditions (no treatment, HGF, and HRG) and a decrease in overall ERBB2 expression (Fig. 4B). This downregulation in RTK expression commonly occurs when the receptor activation is increased. In the ERBB2–KD cells, we observed an increase in MET activity in the HGF-treated cells (Fig. 4A). Therefore, both MET and ERBB2 are able to compensate for the inhibition of the other receptor through increased activation. This compensatory upregulation of activity seemed to be enhanced by ligand stimulation but was not completely dependent on it, especially for ERBB2. These results suggest that breast cancer cells with dual MET and ERBB2 expression are likely to be resistant to targeted therapy toward ERBB2 or MET alone.

EGFR and ERBB3 do not compensate for MET and ERBB2 knockdown in MET+/ERBB2+ breast cancer cells

Even though our results indicated that MET activity is increased when ERBB2 is depleted, it is possible that other receptors are involved in maintaining survival signals in breast cancer cells. The ERBB family members EGFR and ERBB3 each can form heterodimers with ERBB2, promote...
ERBB2+ breast cancers (26, 27), and have been shown to play a role in trastuzumab resistance (28, 29). In addition, EGFR and ERBB3 can promote the resistance of MET–addicted gastric carcinomas to MET inhibitors (23). Therefore, EGFR and ERBB3 are suspects in driving the resistance to ERBB2 or MET knockdown.

To determine whether EGFR or ERBB3 is involved in compensating for MET or ERBB2 depletion in HCC1954 cells, we evaluated their expression and activity in MET–KD and ERBB2–KD cells. Unexpectedly, we observed no change in the expression or activity of ERBB3 and EGFR in MET–KD cells (Fig. 5A and B). In ERBB2–KD cells, a decrease in both P–ERBB3 and P–EGFR was observed. As ERBB2 is the preferred dimerization partner of the ERBB receptors, depletion of ERBB2 likely decreases heterodimerization with ERBB3 and EGFR and the

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**Figure 3.** Quantitation of MET and ERBB2 expression in ERBB2+ and ERBB2− human breast cancers. Quantitation of MET/ERBB2 multispectral imaging was conducted on 30 human breast carcinoma sections to identify MET (green), ERBB2 (red), and colocalized MET/ERBB2 signals (yellow). Three to 4 optical fields were analyzed for each slide. A, in ERBB2− tumors (clinically defined as 0–1 by Hercept analysis), high levels of MET expression were observed with the highest levels existing in the ER−/ERBB2− tumors relative to ER+/ERBB2− tumors. B, MET is expressed in the majority of ERBB2+ breast cancers (clinically defined as 2–3+ by the Hercept test), and coexpression of MET and ERBB2 was commonly present in MET+ tumors. Four ERBB2+ tumors defined as 2+ by the Hercept test were included, but only #65 was determined to be ERBB2 amplified by FISH. Error bars represent SD in positive pixels per region of interest.
subsequent phosphorylation of these receptors. The decrease in ERBB3 and EGFR activity corresponded with upregulation of receptor expression. Collectively, these data show that knockdown of MET results in increased ERBB2 activation and that ERBB2 knockdown leads to increased MET activation and a decrease in EGFR and ERBB3 signaling.

**MET and ERBB2 knockdown result in increased ERK signaling**

It can be argued that tumor plasticity in signaling networks is essential for maintaining two critical survival pathways, PI3K/AKT and MAPK/ERK. We examined both of these pathways after MET or ERBB2 depletion. In the control cells, P-AKT (Ser473) levels were high, but...
both MET and ERBB2 knockdown caused a decrease in AKT activation (Fig. 6A). An increase in MAPK activation was observed with the loss of MET, but P-MAPK increased the most with ERBB2 depletion (Fig. 6B). This supports the observations of Serra and colleagues where PI3K inhibition in ERBB2-overexpressing cells was compensated for by ERK/MAPK dependence (30). We also examined other kinases downstream of the MET and ERBB2 receptors using a phospho-kinase antibody array of 42 kinases, but observed no differences in their activity. This result underscores the plasticity of the signaling networks that these breast cancer cells possess. Knockdown of the MET or ERBB2 receptor is easily compensated for by activation of other RTK signaling networks, and the loss of AKT signaling shifts the equilibrium in survival signals to ERK/MAPK signaling.

**Figure 6.** MET and ERBB2 knockdown result in decreased AKT signaling and increased ERK activity. A, Western blot and densitometry analysis of AKT showed a decrease in P–AKT (Ser473) upon knockdown of either MET or ERBB2. Total AKT expression was unchanged. B, P-MAPK increased upon either MET or ERBB2 knockdown but was pronounced with ERBB2 knockdown. Total MAPK was unchanged. HCC1954 cells were infected with control shRNA, MET shRNA, or ERBB2 shRNA and treated with vehicle, HGF, or HRG for 24 hours. β-Tubulin was used as a loading control. Error bars indicate SD.

**Figure 7.** MET or ERBB2 knockdown alone is insufficient to block proliferation of breast cancer cells. Cell proliferation was measured by [3H]-thymidine incorporation. HCC1954 cells were infected with control shRNA, MET shRNA, or ERBB2 shRNA. Cells were serum-starved and then stimulated with or without HGF (100 ng/mL) or heregulin (10 ng/mL), and proliferation was measured 20 hours after stimulation. Student t test (two-tailed) was used to analyze statistical differences (*, P < 0.05; **, P < 0.001).

**MET or ERBB2 knockdown alone are insufficient to block proliferation in MET⁺/ERBB2⁺ breast cancer cells**

Next, we measured the effect of MET or ERBB2 knockdown on cell proliferation. In cells treated with vehicle, MET and ERBB2 knockdown induced a 30% and 35% reduction in proliferation, respectively (Fig. 7). With HGF treatment, we observed a significant increase in proliferation in the control cells; however, in MET-KD cells, this response was reduced by 67% and in ERBB2–KD cells, by 51%. Heregulin treatment had less of a stimulatory effect on proliferation, and MET-KD and ERBB2-KD cell proliferation decreased by only 29% and 40%, respectively. MET-KD cell proliferation decreased the most in the presence of HGF, whereas ERBB2-KD cell proliferation decreased significantly with HRG treatment. These observations support the fact that the surrounding tumor microenvironment and expression of receptor ligands can have a significant impact on the response of tumor cells in the presence of TKIs. Overall, this data supports the concept that the loss or inhibition of one RTK is insufficient to kill all cells and will only decrease proliferation in a subpopulation of susceptible cells.
Discussion

We examined the intratumoral heterogeneity of RTK expression in breast cancer and its influence on signaling robustness and therapeutic resistance. The RTKs MET, ERBB2, and EGFR are known to be highly expressed in aggressive breast cancer subtypes. In addition, compensatory signaling of MET and the ERBB family is linked to resistance to TKIs in other cancers, including lung, gastric, and colon carcinomas. To elucidate the relationship between MET and ERBB2 in breast cancer, we analyzed the expression of MET and ERBB2 in human breast cancers and how the loss of MET or ERBB2 in MET+/ERBB2+ breast cancer cells affects RTK signaling networks and proliferation. Costaining of human breast cancers with MET and ERBB2 revealed that the majority of ERBB2+ breast cancers express heterogeneous patterns of MET and ERBB2. Accordingly, these tumors contained variable levels of MET+/ERBB2+, MET+/ERBB2−, and MET+/ERBB2+ subpopulations. Because the expression of multiple RTKs is associated with innate and acquired resistance to TKIs, we examined whether either MET or ERBB2 is able to compensate for the inhibition or knockdown of the other in HCC1954 breast cancer cells that highly express both MET and ERBB2. Interestingly, we observed that MET knockdown increased ERBB2 activation and conversely, ERBB2 knockdown increased MET activation. Activation of EGFR and ERBB3 was not observed after MET or ERBB2 depletion. This suggests that in MET+/ERBB2+ breast cancers, targeted inhibition of MET or ERBB2 will be compensated for by activation of the remaining RTK. We also observed a shift in downstream signaling: the loss of MET or ERBB2 led to a decrease in PI3K/AKT signaling and an increase in ERK/MAPK activation. Given that these changes were measured 24 hours after stimulation, earlier timepoints may reveal variations in Akt activity. MET or ERBB2 knockdown did decrease proliferation but neither alone was able to abolish all cell growth. These results suggest that plasticity in kinase signaling supports tumor growth during therapeutic resistance.

The high level of inter- and intratumoral heterogeneity that is present in breast cancers suggests that each patient’s heterogeneity profile needs to be considered in determining treatment strategies. Even in the ERBB2+ breast cancer subtype, there are several known mechanisms of trastuzumab resistance. For instance, patients with PTEN mutations will require a therapeutic combination targeting ERBB2 and the PI3K/AKT pathway. Our study shows there is a subset of ERBB2+ breast cancers that express MET and contain MET+/ERBB2+ subpopulations. These data and the analysis of RTK activation during ERBB2 knockdown indicate that MET signaling is a compensatory pathway of resistance. These results are supported by Shattuck and colleagues, who observed an increase in MET expression with trastuzumab treatment of ERBB2-dependent cell lines (31). In addition, the expression of multiple RTKs in other cancers has been shown to promote resistance through ligand activation or upregulation (6, 7). Therefore, ERBB2+ breast cancers with MET+/ERBB2+ subpopulations may have an innate resistance to ERBB2 inhibition and may benefit from combined MET and ERBB2 inhibition. In addition, breast cancers that are MET-dependent may be resistant to MET inhibition via ERBB2 activation and require combined MET and ERBB2 inhibition.

The interaction between MET and ERBB receptors is likely to be important in several cancers including several breast cancer subtypes. In this study, we show a role for MET in ERBB2+ breast cancers. The high expression of EGFR and MET in triple-negative breast cancers indicates that MET and the ERBB receptor family may drive resistance to TKIs in other breast cancer subtypes. The expression of several RTKs in a tumor ensures activation of multiple signaling pathways, including PI3K/AKT and ERK/MAPK, to maintain the signaling plasticity necessary for tumor progression and resistance. Therefore, it is critical that for each breast cancer subtype, we identify biomarker panels that identify potential mechanisms of resistance. These biomarkers of molecular heterogeneity may be used as both diagnostic and prognostic measures of metastatic potential and treatment sensitivity or resistance. Moreover, profiles of intratumoral heterogeneity will assist clinicians in tailoring effective treatment combinations targeting multiple RTK or other signaling pathways in patients with breast cancer.

Disclosure of Potential Conflicts of Interest

C.R. Gravel has commercial research support from Novartis. No potential conflicts of interest were disclosed by the other authors.

Dedication

This publication is dedicated to Debra Bauters and her courageous fight against cancer.

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