Proliferation of Colorectal Cancer Is Promoted by Two Signaling Transduction Expression Patterns: ErbB2/ErbB3/AKT and MET/ErbB3/MAPK

Yong-Liang Yao¹, Jie Shao³, Chunfu Zhang⁴, Jian-Hong Wu¹, Qing-Hui Zhang¹, Jian-Jun Wang¹, Wei Zhu²*

¹Department of Clinical Laboratory, Kunshan First People’s Hospital, Affiliated to Jiangsu University, Kunshan, Jiangsu, China, ²School of Medical Science and Laboratory Medicine, Jiangsu University, Zhenjiang, Jiangsu, China, ³Department of Clinical Laboratory, The 359th Hospital of PLA, Zhengjiang, Jiangsu, China, ⁴Department of Clinical Laboratory, The Second People’s Hospital of Kunshan, Kunshan, Jiangsu, China

Abstract

One of the recent breakthroughs in cancer research is the identification of activating mutations in various receptor tyrosine kinase (RTK) pathways in many cancers including colorectal cancer (CRC). We hypothesize that, alternative to mutations, overexpression of various oncogenic RTKs may also underpin CRC pathogenesis, and different RTK may couple with distinct downstream signaling pathways in different subtypes of human CRC. By immunohistochemistry, we show here that RTK members ErbB2, ErbB3 and c-Met were in deed differentially overexpressed in colorectal cancer patient samples leading to constitutive activation of RTK signaling pathways. Using ErbB2 specific inhibitor Lapatinib and c-Met specific inhibitor PHA-665752, we further demonstrated that this constitutive activation of RTK signaling is necessary for the survival of colorectal cancer cells. Furthermore, we show that RTK overexpression pattern dictates the use of downstream AKT and/or MAPK pathways. Our data are important additions to current oncogenic mutation models, and further explain the clinical variation in therapeutic responses of colorectal cancer. Our findings advocate for more personalized therapy tailored to individual patients based on their type of RTK expression in addition to their mutation status.

Citation: Yao Y-L, Shao J, Zhang C, Wu J-H, Zhang Q-H, et al. (2013) Proliferation of Colorectal Cancer Is Promoted by Two Signaling Transduction Expression Patterns: ErbB2/ErbB3/AKT and MET/ErbB3/MAPK. PLoS ONE 8(10): e78086. doi:10.1371/journal.pone.0078086

Editor: Jin Q. Cheng, H.Lee Moffitt Cancer Center & Research Institute, United States of America

Received: June 14, 2013; Accepted: September 7, 2013; Published: October 30, 2013

Copyright: © 2013 Yao et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Social Development Foundation of Kunshan (no. KS1130) and the Scientific Research Foundation of Jiangsu University (no. 11JDG0090). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: zhuwei@ujs.edu.cn

Introduction

Colorectal cancer is an epithelial malignancy occurring in the colon or rectum. More than 1 million people are diagnosed with colorectal cancer yearly worldwide resulting in about 0.5 million deaths. In 2008, it was the second most common cause of tumor-associated death in women and the third most common in men. It is more common in developed than in developing countries [1–3]. Chemotherapy may be used as adjuvant therapy in addition to surgery in almost all colorectal cancer patients [4–6]. With or without lymph node metastasis, chemotherapy is considered to increase life expectancy and reduce the possibility of recurrence. Chemotherapy drugs are designed to act via a variety of mechanisms and may include combinations of agents such as fluorouracil, capecitabine, UFT, leucovorin, irinotecan, or oxaliplatin [7–9]. However, for various reasons, chemotherapy can cause other problems for the patients mainly due to its non-specific attack and related side effects, such as nausea, vomiting, hair loss, fatigue, anemia and infection among others. Therefore, targeted cancer therapy has emerged which has been developed based on knowledge of cell signaling. Targeted cancer therapy mainly depends on small-molecule drugs and monoclonal antibodies which cause far fewer side effects compared to traditional chemotherapy.

Exogenous signals such as epidermal growth factor (EGF) and hepatocyte growth factor (HGF) were previously reported to be vital to maintain sustained growth of tumor cells by binding to their receptors. These receptors belong to the receptor tyrosine kinases (RTKs) family and ideal targets in cancer therapy as demonstrated in many studies. After systemic profiling of cancer genomes by using the next generation sequencing, we now know that acquisition of activating mutations in RTKs or downstream signaling molecules is one of the major oncogenic mechanisms. However, only a small subset of patients possess these mutations, suggesting that other alternative mechanisms are at play. One of these alternative mechanisms could be the overexpression of pro-proliferation and pro-survival RTKs, and/or differential usage of downstream signaling pathways. With more in-depth analysis of cancer genomes, we are now begin to understand the individual differences which signify unique disease biology and clinical management requirement.

The family of epidermal growth factor receptor (EGFR) and c-Met are RTKs that have been frequently reported in colorectal carcinoma progression and metastasis. Activation of these RTKs can stimulate a number of specific pathways directly effecting tumor cell migration, survival and proliferation. The aberrant regulation of the RTKs is often noted in advanced CRC. Compared to EGFR, other EGFR family members, c-MET,
ErbB2, ErbB3, and their related signaling pathways are relatively unknown [10]. Therefore, in the present study, we investigated the differential expression of RTKs c-MET, ErbB2, ErbB3, and downstream signaling molecules in primary samples of colorectal cancer patients, as well as in representative human colorectal cancer cell lines.

Materials and Methods

Patients

A total of 105 colorectal cancer specimens were investigated in this study and were obtained from patients at the time of surgical resection and endoscopy. Normal colorectal tissues were obtained from 40 Chinese patients with non-tumoral diseases such as tediously long colorectal and vascular malformation. All of the cases were treated at the First People’s Hospital of Kunshan, China between January 2010 and August 2011, and all the samples were collected according to the principles expressed in the Declaration of Helsinki. Written documented informed consent for gene expression analyses of all tissues was obtained from all patients prior to surgery or endoscopy examination. This study and the consent procedure were approved by the local ethics committee of the First People’s Hospital of Kunshan, China. The diagnosis and staging of colorectal cancer was assessed according to the AJCC (TNM) Staging System.

Cell Culture and Reagents

LoVo, SW948 and SW480 cell lines were obtained from ATCC. EGF was from Sigma (Milan, Italy), HRG1-β1 and IGF-1 from R&D Systems, [Minneapolis, MN]. LY294002 was from
Sectioned slides were stained immunohistochemically for ErbB2, ErbB3 and c-MET (Santa Cruz Biotechnology, Inc.) followed by incubation with secondary antibody at 37°C for 30 min and reaction with DAB reagent for 5–10 min. The slides were mounted with neutral gum for microscopic examination. Cells with brown intracellular granules (cytoplasm or nucleus) were considered to be positively stained.

**Cell Viability**

Cultured cells were plated at a density of $6 \times 10^5$ cells/well in a 96-well plate and treated with various agents. Cell viability was evaluated by an MTS assay. CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI) was added to each well according to the manufacturer’s instructions, and the plates were returned to the incubator. After 4 hours, cell viability was determined by measuring the absorbance at 490 nm using a computer controlled plate-reader.

**Statistical Analysis**

Data are expressed as mean ± standard deviation (SD). Comparisons between two groups were performed using Student’s t-test or the Mann-Whitney U test, as appropriate. All statistical analyses were performed using SPSS statistical software (version 13.0), and two-tailed t-tests were applied to all data unless otherwise specified. A value of $P$ less than 0.05 was considered to be statistically significant.

**Results**

**Differential Expression of ErbB3, ErbB2 and c-MET in Human Colorectal Cancer**

In order to investigate the expression of a set of receptor tyrosine kinase genes (RTK) in human colorectal cancer, paraffin embedded blocks were collected which contained specimens of 105 cases of colorectal cancer. First, the expression of the important oncogenic heterodimer ErbB2/ErbB3 was determined by IHC. As shown in Figure 1A, ErbB3 was significantly overexpressed in human colorectal cancer tissue, with expression mainly distributed in the membrane of cancer cells. The partner of ErbB3 in the heterodimer, ErbB2, was also located mainly in the membrane of cancer cells and was similarly overexpressed in human colorectal cancer (Figure 1B). Another receptor tyrosine kinase, the proto-oncogene C-MET, was also detected by IHC, in the membrane of cancer cells in most cases (Figure 1C). Finally, quantitative analysis of expression of the three proteins was performed using the image analysis software Image-pro plus. The IHC-positive areas from five random fields of each IHC-stained section were evaluated as integrated optical density (IOD). All three proteins were significantly overexpressed in human colorectal tissues. (ErbB3: cancer tissues 3264±1032 vs. normal colorectal tissues 1038±354, $P=0.0074$, ErbB2: cancer tissues 2616±2019 vs. normal colorectal tissues 338±154, $P=0.016$, c-MET: cancer tissues 3812±739 vs. normal colorectal tissues 978±313, $P=0.0057$ by Mann-Whitney U test).

The results of expression of all three proteins were expected, not only due to their role as proto-oncogenes, but also because previous similar studies have indicated similar results. However, after further investigation, we found varying degrees of variation in the expression of these three proteins in human colorectal cancer, especially in ErbB2 expression. Of the 105 cases examined, 45 cases (42.85%) were negative for ErbB2 staining, 8 cases showed strong ErbB2 staining, and the remainder (52 cases) showed moderate or weak ErbB2 staining. Only 9 cases (8.5%) were negative for c-MET staining while 14 cases exhibited strong

**Immunohistochemistry (IHC)**

All tissues were removed and fixed in 4% paraformaldehyde overnight at 4°C, then processed and sectioned at 5 μm thickness.
staining and 82 cases showed moderate or weak c-MET staining. Interestingly, ErbB3 was positive in all colorectal cancer tissues examined (Figure 2A). Moreover, the heterogeneous expression of these three proteins in four colorectal cancer tissues was further investigated by western-blot (Figure 2B). The difference in expression between cancer tissues and normal tissues of all three proteins confirmed the reliability of IHC staining, and further indicated that three types of expression patterns existed in the 105 cases. All the cases examined could be classified as: ErbB3: c-MET co-over-expression colorectal cancer, ErbB3:ErbB2 co-over-expression colorectal cancer, and ErbB3:ErbB2: c-MET co-over-expression colorectal cancer (Figure 2C). We believe that these results are a good example of variation of tumor RTK expression pattern, and furthermore, that the molecular mechanisms underlying the different patterns are worthy of exploration.

Proliferation of Colorectal Cancer Cells is Mediated by Either ErbB3/ErbB2 or ErbB3/c-MET Signaling Pathways

In order to identify suitable human colorectal cancer cell lines corresponding to the three molecular patterns which we discovered in the human samples, the expression of ErbB2, ErbB3 and c-MET in 9 human colorectal cancer cell lines was analyzed by western blotting (data not shown). The result showed that SW480 is a representative cell line co-expressing ErbB2, ErbB3 and c-MET. The cell line LoVo only co-over-expresses ErbB2 and ErbB3, while SW48 is a representative cell line which co-over-expresses ErbB3 and c-MET (Figure 3A). Cell viability was accessed by MTS assay, and the related mechanisms will be further discussed. Some inhibitors and agonists of these three proteins were selected including PHA-665752 (PHA) and lapatinib, which are inhibitors of c-MET and epidermal growth factor receptor (EGFR), respectively. Once c-MET signaling was blocked by PHA, the cell viability of SW480 and SW948 decreased significantly (Figure 3 B and D); however no significant change in cell viability occurred in LoVo cells, perhaps due to their low expression of c-MET (Figure 3C). Conversely, lapatinib, an ErbB2 inhibitor, significantly reduced the cell viability of LoVo and SW480 cells, while having almost no effect on SW948 cells which have low ErbB2 expression (Figure 3D). The differences between LoVo and SW948 cells in their response to PHA and lapatinib are possibly due to differences in their signal transduction pattern (Figure 3 C and D). Moreover, in order to investigate whether the agonists can rescue cell viability, HGF, an agonist of c-MET, and heregulin-β1 (HRG1-β1), which binds to HER3 and induces its heterodimerization with the other family members, were used. Both HGF and HRG1-β1 could partially rescue the viability of SW480 cells (Figure 3B), however, the situation was different in LoVo and SW948 cells. HGF stimulation caused an insignificant increase of cell viability in LoVo cells compared with LoVo cells treated with PHA (Figure 3C).
SW948 cells responded more sensitively to HGF, with a significant increase in cell viability compared to PHA-treated SW480 cells (Figure 3D). However SW948 cells responded less sensitively to HRG1-β1 compared to LoVo, perhaps due to their low expression of ErbB2, but the cell viability of SW948 was still increased, possibly due to the other receptors of the EGFR family such as EGFR and ErbB4 which can both dimerize with ErbB3. Therefore, different responses were explored due to different molecular patterns of expression: ErbB3/ErbB2 and ErbB3/c-MET. The downstream signaling was then investigated further.

Different Molecular Patterns of Colorectal Cancer Involve the Activation of Downstream MAPK or AKT Signaling

In order to investigate further the mechanism of downstream signaling of both molecular patterns in colorectal cancer, cell lysates were utilized to analyze the activation of AKT and MAPK signaling which are well-known downstream signaling pathways of ErbB2/ErbB3 and ErbB3/c-MET complexes. An inducible siRNA system was involved in the study, and the expression of ErbB3 could be silenced by doxycycline (DOXY) treatment. Once SW948 was treated with PHA or DOXY, activation of both AKT and MAPK were blocked. Both these two signaling pathways could be reactivated by HGF, but no significant reactivation was found when ErbB3 was silenced even with HGF treatment, indicating that activation of MAPK signaling in SW948 depended more on ErbB3 (Figure 4A). The activation of AKT and MAPK signaling was accessed in a different way in LoVo cells due to their high expression of ErbB2/ErbB3. Both AKT and MAPK signaling were effectively blocked when treated with DOXY and lapatinib, indicating impairment of both ErbB3 and other EGFR molecules which can inactivate AKT and MAPK signaling. HRG1-β1 was able to strongly activate both AKT and MAPK signaling; however its activation effect on AKT was more dependent on ErbB3 while its activation effect on MAPK was more dependent on other EGFR family members (Figure 4B).

Furthermore, the treatment conditions are reversed between these two cells. Lapatinib has little effect on activation in SW948 cells, but HRG1-β1 can still induce activation of both AKT and MAPK signaling although the activation degree is much weaker than in LoVo cells (Figure 4C). Similarly, the c-MET inhibitor PHA alone has almost no effect on AKT and MAPK signaling, and HGF can barely activate either AKT or MAPK signaling probably due to its low expression of c-MET (Figure 4D). The specificity of the growth signaling via ErbB3/ErbB2 or ErbB3/c-MET was further studied using specific inhibitors.

Figure 4. Activation of MAPK or AKT signaling in colorectal cancer cells with different molecular patterns of expression. (A, B) Detection of AKT and MAPK signaling in SW948 and LoVo cells by western-blot, with the treatment indicated in the figure. (C, D) Detection of AKT and MAPK signaling by western-blot in LoVo and SW948 cells with HGF or HRG1-β1 treatment, with or without different agonists or antagonists, as indicated.
doi:10.1371/journal.pone.0078086.g004
AKT and MAPK are Two Cell-signaling Pathways Governing Cell Viability of Colorectal Cancer Cell Lines with Different Receptor Expression Patterns

In order to further investigate the RTK expression variation in colorectal cell lines, a series of tests of cell viability and cell signaling pathways were performed using specific inhibitors of AKT and MAPK. For LoVo cells, cell viability was significantly decreased when treated with LY294002, an inhibitor of AKT signaling. However, there was no significant effect when it was treated with an inhibitor of c-MET, U0126. If LoVo was treated with both inhibitors, the rescue effect of HRG1-β1 on cells was much stronger than that of HGF (Figure 5A), and signaling activation analysis by western blot also implied that activation of both AKT and MAPK was partially restored by HRG1-β1 even when treated with inhibitors (Figure 5B). This result indicated that cell viability was not very dependent on ErbB3/c-MET/MAPK signaling but instead depended on ErbB3/ErbB2/AKT signaling. However, the situation is not the same in SW948 cells, in which the impairment of cell viability by the inhibitor of c-MET, U0126, was stronger than that caused by LY294002, and the restorative effect was different from that in LoVo cells when treated with both LY294002 and U0126, with the restorative effect of HGF being stronger than HRG1-β1, and the activation of both AKT and MAPK signaling being more strongly induced by HGF than HRG1-β1 (Figure 5C, D). Taken together, our results show that colorectal cancer exhibits variation in oncogenic RTK due to different molecular patterns of expression including ErbB2/ErbB3/AKT and ErbB3/c-MET/MAPK.

Discussion

Activation of RTKs is critical for the proliferation and survival of normal and malignant cells. Recently, various RTK activating mutations have been identified in a subset of cancer patients by next generation sequencing, suggesting that constitutive activation
of RTKs is one of the underpinning mechanisms of cancer development. However, the remaining population of cancer patients possess no such mutations implying that alternative RTK activating mechanisms exist. Here we show one of such alternative mechanisms is the overexpression of the RTK family members of c-MET, and ErbB2, ErbB3, in CRC cells as well as representative CRC cell lines.

C-MET is one of the receptor tyrosine kinases (RTK) which are widely distributed on the surface of epithelial-endothelial originating cells. Its only known ligand, hepatocyte growth factor (HGF), is expressed mainly in cells of mesenchymal origin [11]. C-MET dimerizes and autophosphorylates upon ligand binding, which in turn creates active docking sites for proteins that mediate serial downstream signaling activation including MAPK, PI3K-AKT, SRC and STAT. Numerous studies have reported that c-MET is overexpressed in a variety of carcinomas including lung, breast, ovary, kidney, colorectal, thyroid, liver, and gastric carcinomas [12–14]. It is well known that c-MET plays important roles in cancer development through its proliferation- and angiogenesis-promoting effects exerted by downstream oncogenic pathways, and metastasis-promoting effect which is mainly dependent on metalloprotease production. Due to its role in oncogenesis and cancer progression, c-MET is considered to be an important target in anticanccer therapy, and some biological antagonists or monoclonal antibodies targeting c-MET have emerged which are known as c-MET TKI [15–18]. Certain gastric cancer and NSCLC cell lines display exquisite sensitivity c-MET TKI, but, cells/tumors treated with c-MET TKI eventually develop resistance. This resistance to c-MET TKI is a result of overcoming the inhibition through sustained high MAPK and PI3K/AKT activity, and EGFR family members can act as complementary receptors for MAPK and PI3K/AKT activation while c-MET is blocked[19–22]. While acquisition of novel mutations may be one of the possible explanations to this resistance in clinical treatment, activating mutations of several RTKs including ErbB2, ErbB3 and c-MET were discovered in human colorectal cancer and other cancers [23–27]. RTK overexpression and downstream signal activation provide an alternative and complimentary mechanism. However, the variability of oncogenic RTK expression levels may also underscore the significance of individualized cancer therapy.

In the present study, we not only confirmed the over-expression of c-MET, ErbB2 and ErbB3 in human colorectal cancer, but also revealed the variation and complementary of these receptors. Moreover, we identified in vivo cell line models with different molecular expression patterns which show differing sensitivity to the treatment of both antagonists and agonists of c-MET and EGFR signaling.

The ErbB2 gene is also commonly referred to as Her-2/neu, one of the growth factor receptors leading cell signaling related to cell proliferation, adhesion and movement [28,29]. It is also a hot target for signaling-based drug development. Trastuzumab, one of the related drugs targeting ErbB2, binds ErbB2 and induces uncoupling of ligand-independent HER2–HER3 heterodimers, thus inhibiting downstream signaling. However, several studies have revealed that trastuzumab treatment can induce resistance both by amplification of signaling through other ErB receptors and by cross-talk with heterologous RTKs [30–32]. As we report in the present study, c-MET has also been found to be upregulated in trastuzumab-resistant ErbB2-overexpressing cells following exposure to trastuzumab. Furthermore, a mechanistic study revealed that activation of c-MET protects cells against trastuzumab by abrogating the induction of p27. This is consistent with the outcome obtained in our study, showing that different molecular patterns exist in human colorectal cancer. Heterogeneous expression of ErbB2 and ErbB3 with varying combinations of RTKs implied potentially different chemotherapy projects.

It is anticipated that an increasing number of molecular signaling pathway-based drugs will emerge, aimed at a variety of targets; however, it is very possible that none of these drugs alone will have a dominant anti-tumor effect. Combination therapy for cancer is an on-going and developing concept and importantly, personal detection and analysis of an individual’s molecular expression pattern is indispensable for more effective therapy with fewer side effects.

**Author Contributions**

Conceived and designed the experiments: YLY WZ. Performed the experiments: YLY JS CZ. Analyzed the data: WZ YLY JHW. Contributed reagents/materials/analysis tools: QHZ JJW. Wrote the paper: WZ YLY.

**References**

1. Ballard-Barbash R, Friedereich CM, Coursney KS, Siddiqi SM, McTiernan A, et al. (2012) Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst 104: 815–840.

2. Benson AB, . (2013) Metastatic colon cancer, version 3.2013: featured updates to the NCCN Guidelines. J Natl Compr Canc Netw 11: 141–152; quiz 152.

3. Romagnolo DF, Selmin OI (2012) Flavonoids and cancer prevention: a review. Nutr Rev 70: S1–S22.

4. Cormont A (2012) Preoperative chemotherapy for colon cancer is getting closer. J Rev Clin Oncol 9: 314–326.

5. Huang S, Holzel M, Knijnenburg T, Schlicker A, Roepman P, et al. (2012) MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. Cell 151: 937–950.

6. Aparicio LA, Valladares M, Blanco M, Alonso G, Figueroa A (2012) Biological pathway in cancer therapy. Expert Opin Ther Targets 16: 553–572.

7. Peters S, Adjei AA (2012) MET: a promising anticancer therapeutic target. Nat Rev Clin Oncol 9: 314–326.

8. Benson AB, . (2013) Metastatic colon cancer, version 3.2013: featured updates to the NCCN Guidelines. J Natl Compr Canc Netw 11: 141–152; quiz 152.

9. Yue P, Zhang X, Paladino D, Sengupta B, Ahmad S, et al. (2012) Hyperactive Lefebvre J, Ancot F, Leroy C, Muharram G, Lemiere A, et al. (2012) Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst 104: 815–840.

10. Jun JI, Lau LF (2011) Taking aim at the extracellular matrix: CCN proteins as therapeutic targets; however, it is very possible that none of these drugs alone will have a dominant anti-tumor effect. Combination therapy for cancer is an on-going and developing concept and importantly, personal detection and analysis of an individual’s molecular expression pattern is indispensable for more effective therapy with fewer side effects.
21. Fan W, Tang Z, Yin L, Morrison B, Hafez-Khayyata S, et al. (2011) MET-independent lung cancer cells evading EGFR kinase inhibitors are therapeutically susceptible to BH3 mimetic agents. Cancer Res 71: 4494–4505.

22. Zhang L, Virani S, Zhang Y, Bhojani MS, Burgess TL, et al. (2011) Molecular imaging of c-Met tyrosine kinase activity. Anal Biochem 412: 1–8.

23. Muzny DM, Bainbridge MN, Chang K, Dinh HH, Drummond JA, et al (2012) Comprehensive molecular characterization of human colon and rectal cancer. Nature 487: 339–357.

24. Bentivegna S, Zheng J, Namsaraev E, Carlton VE, Pavlicek A, et al. (2008) Rapid identification of somatic mutations in colorectal and breast cancer tissues using mismatch repair detection (MRD). Hum Mutat 29: 441–450.

25. Jaiswal BS, Kljavin NM, Stawiski EW, Chan E, Parikh C, et al. (2013) Oncogenic ERBB3 mutations in human cancers. Cancer Cell 23: 603–617.

26. Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, et al. (2005) Colorectal cancer: mutations in a signalling pathway. Nature 436: 792.

27. Wood LD, Parsons DW, Jones S, Lin J, Szklom T, et al (2007) The genomic landscapes of human breast and colorectal cancers. Science 318: 1108–1113.

28. Gajria D, Chandarlapaty S (2011) HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther 11: 263–273.

29. Gutierrez C, Schiff R (2011) HER2: biology, detection, and clinical implications. Arch Pathol Lab Med 135: 55–62.

30. Chow A, Arteaga CL, Wang SE (2011) When tumor suppressor TGFbeta meets the HER2 (ERBB2) oncogene. J Mammary Gland Biol Neoplasia 16: 61–68.

31. Miller TW, Balko JM, Arteaga CL (2011) Phosphatidylinositol 3-kinase and antioestrogen resistance in breast cancer. J Clin Oncol 29: 4452–4461.

32. Osborne CK, Schiff R (2011) Mechanisms of endocrine resistance in breast cancer. Annu Rev Med 62: 233–247.