ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 2/2023
261–269
https://doi.org/10.18388/abp.2020_6357

Regular paper

β-catenin promotes resistance to trastuzumab in breast cancer cells through enhancing interaction between HER2 and SRC

Xiaoyan Hao[1], Jialu Zheng[2], Xiaoqin Yu[3], Zhixin Li[1] and Guanghui Ren[4]✉

[1]Department of Thyroid, Breast Surgery, Shenzhen Traditional Chinese Medicine Hospital, Shenzhen, China; [2]Department of Thyroid, Breast Surgery, Longgang District Central Hospital, Shenzhen, China; [3]Department of Ultrasound, Longgang District Central Hospital, Shenzhen, China; [4]Department of General Surgery, Shenzhen Hospital, Southern Medical University, Baoan District, Shenzhen, Guangdong, China

More than 1 million women worldwide are diagnosed with breast cancer (BC) each year. This study aims to explore the molecular mechanisms of β-catenin affecting the trastuzumab tolerance in HER2-positive BC. β-catenin in BC and non-BC tissue samples were assessed by immunohistochemistry. β-catenin and HER2 were over-expressed and knockdown to evaluate their role in tumorigenicity and trastuzumab resistance in cell and animal models using soft-agar and xenograft assays. Confocal laser immunofluorescence assay and co-immunoprecipitation were used to assess protein-protein binding. Expression of genes was detected using Western blot analysis. β-catenin was highly expressed in primary and metastatic BC, overexpression of β-catenin increased the colony formation of MCF7 cells when it was co-expressed with HER2 and synergically increased the tumor size in immunodeficient mice. Overexpression of β-catenin also increased the phosphorylation of HER2 and HER3 and increased the size of tumor derived from HER2-elevated cells. Confocal laser immunofluorescence assay showed that β-catenin and HER2 were co-localized on the membrane of MDA-MB-231 cells, suggesting that β-catenin binds HER2 to activate the HER2 signaling pathway. Immunoprecipitation of β-catenin and HER2 also confirmed this binding. On the other hand, knockdown of β-catenin in MDA-MB-231 cell lines decreased the activity of SRC and decreased phosphorylation of HER2 at Y877 and Y1248. The interaction between HER2 and SRC was enhanced when β-catenin was overexpressed, and β-catenin increased the resistance of tumor derived from HER2 elevated BT474 cells to trastuzumab. Further analysis showed that trastuzumab inhibited the activation of HER3, but SRC was still highly expressed in cells overexpressing β-catenin. Our work demonstrates that β-catenin is highly expressed in BC and it synergically promotes formation and progress of BC with HER2. β-catenin binds with HER2 leading to enhanced interaction with SRC and resistance to trastuzumab.

Keywords: β-catenin; HER2-positive BC; trastuzumab; drug resistance

Received: 10 May, 2022; revised: 06 August, 2022; accepted: 11 January, 2023; available on-line: 17 April, 2023

✉e-mail: guanghuirendr@yeah.net

Abbreviations: Akt, protein kinase B; ANOVA, analysis of variance; BC, breast cancer; DAB, diaminobenzidine; DMEM, Dulbecco's modified Eagle medium; ECL, enhanced chemiluminescence; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EMEM, Eagle's minimum essential medium; ERK, extracellular si-gnal-regulated kinase; FDA, Food and Drug Administration; GBR2, growth factor receptor-bound protein 2; HER2, human epidermal growth factor receptor 2; MAPK, mitogen-activated protein kinase; MOI, multiplicity of infection; PI3K, phosphoinositide 3-kinase; PVDF, polyvinylidene difluoride; SD, standard derivation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SRC, tyrosine kinase; VEGF, vascular endothelial growth factor

INTRODUCTION

Over one million women are diagnosed with breast cancer (BC) every year worldwide. Although the overall survival rate of BC has been greatly improved in the last decade, more than 450,000 people still die of BC each year (Anastasiadi *et al*., 2017; Kolak *et al*., 2017; Peairs *et al*., 2017). Therefore, better understanding of the occurrence and development of BC is essential for clinical development for effective interventions (Fahad Ullah, 2019; Ganz & Goodwin, 2015). In the 1980s, the human epidermal growth factor receptor 2 (HER2) signaling pathway was found to be responsible for the abnormal proliferation of HER2 positive (HER2+) BC (Escriva-de-Romani *et al*., 2018; Rajarajan *et al*., 2020). HER2 is highly expressed in more than 20% of metastatic BCs and is associated with cancer metastasis and poor patient prognosis (Lewis Phillips *et al*., 2008; Smith *et al*., 2007).

HER2 is a member of the epidermal growth factor receptor family (ErbB family) and is activated by homo- or heterodimerization to promote phosphorylation of monomers and recruitment of receptor complexes, phosphoinositide 3-kinase (PI3K), SHC, and growth factor receptor-bound protein 2 (GBR2) (Dankort *et al*., 2001; Ellis & Ma, 2019; Xie & Hung, 1996). Phosphorylated HER2 can activate the PI3K/AKT and MAPK/ERK signaling pathways to promote cell proliferation, survival and differentiation (Lee *et al*., 2013; Ruiz-Saenz *et al*., 2018). HER2$^+$ BC is clinically very aggressive and a series of HER2-targeting drugs, such as trastuzumab, lapatinib, pertuzumab and T-DM1 have been developed for clinical treatment of BC with good clinical efficacy and significantly improved outcomes (Cameron *et al*., 2017a; Derakhshani *et al*., 2020; Hunter *et al*., 2020; Rinnerthaler *et al*., 2019; Saura *et al*., 2020). However, metastatic HER2$^+$ BC remains an incurable disease and new treatment options are required for better treatment (Bredin *et al*., 2020; Harbeck *et al*., 2019). Furthermore, drug resistance, especially trastuzumab resistance, in BC, particularly in HER2$^+$ BC, has eventually emerged in the great majority of treated patients (Nahta *et al*., 2006). A better understanding of mechanisms underlying the drug resistance in HER2$^+$ BC is therefore critical to develop new treatment strategies and drugs.

Trastuzumab is a Food and Drug Administration (FDA)-approved humanized antibody targeting HER2. It is used in combination with chemotherapy drugs to treat HER2$^+$ BC and has a good response in early-stage

BC (Cameron *et al.*, 2017b; Tolaney *et al.*, 2021). Paclitaxel, trastuzumab, and pertuzumab are recommended as first-line therapy for patients with HER2+ BC who have not received trastuzumab or who have metastasized after 6 months of adjuvant trastuzumab therapy (Santa-Maria *et al.*, 2016). However, if metastases occur while receiving trastuzumab, T-DM1, may be the best option (Ramagopalan *et al.*, 2021; Uijen *et al.*, 2022). For example, in the CLEOPATRA clinical trial, patients received T-DM1 as a second-line therapy after receiving a first-line chemotherapy regimen (Larionov, 2018). In addition to T-DM1, lapatinib in combination with chemotherapy and trastuzumab is also a standard chemotherapy regimen, although the sequence of these agents has not been fully defined. Due to the development of drug tolerance in patients receiving trastuzumab, numerous studies and clinical trials have attempted to explore new ways to target the HER2 receptor where the Wnt/β-catenin signaling pathway plays an important role (Schade *et al.*, 2013). Therefore, identifying regulators targeting this pathway may provide new directions and strategies for BC treatment.

Wnt/ β-catenin signaling pathway is involved in the development of early embryos and the occurrence of diseases. It plays an important role in thermogenesis and can promote the abnormal proliferation of tumor cells (Wu *et al.*, 2012; Wu *et al.*, 2017). More than 20 target genes have been identified in the pathway and most of them are related to cell proliferation and occurrence of tumors, such as axin-2 (Doumpas *et al.*, 2019), c-myc, cyclin D1 and VEGF (Liu *et al.*, 2016; Zhang *et al.*, 2012). Studies have shown that Wnt/β-catenin signaling pathway is abnormally activated in human cancers, including BC (Brennan & Brown, 2004; Song *et al.*, 2016). Alternation of β-catenin expression may result from mutation and amplification, both of them lead to the occurrence and progression of BC (Sefidbakht *et al.*, 2021; Zhang *et al.*, 2021). Because β-catenin plays an important role in the formation and metastasis of tumor formation, small molecule inhibitors that target β-catenin are being developed to suppress tumor progression (Wang *et al.*, 2021). Studies have found that in BC cells with high expression of HER2 and trastuzumab resistance, the Wnt/β-catenin signaling pathway is highly activated, suggesting that β-catenin plays an important role in HER2 signaling pathway (Hsieh *et al.*, 2016; Mir *et al.*, 2020; Shen *et al.*, 2020). However, how β-catenin promotes resistance to trastuzumab in BC is largely unclear.

This study aimed to explore the molecular mechanism of β-catenin affecting the trastuzumab tolerance in HER2-positive BC. The findings would provide new directions and potential targets for clinical treatment of HER2+ BC.

MATERIALS AND METHODS

Patients and tissue samples

Tissue samples were collected from patients undergoing surgical treatments at our hospital between April 2013 and September 2017. Patients were female, aged between 32 and 55 years with primary BC that was histologically or cytologically proven. Patients were included if they were HER-2 positive based on assessments from certified laboratories, with ≤1 Eastern Cooperative Oncology Group Performance Status (ECOG PS) and ≥3 month predicted survival and at least one measurable lesion based on RECIST1.1. Patients were excluded if they were male, had incomplete clinical and pathological data (including immunohistochemical), HER2 status was not verified with FISH, had recurrent BC, or died of non-tumor cause during follow-up.

This study was approved by the ethics committee of the Shenzhen Hospital Affiliated with Southern Medical University and carried out in accordance with the declaration of Helsinki (revised in Tokyo in 2004). Written informed consent was obtained from every patient.

Reagents and equipment

Herceptin (trastuzumab) was purchased from Roche, USA. 0.25% trypsin solution (cat no. 25200072), BCA protein assay kit (cat no. 23225), SDS-PAGE sample prep kit (cat no. 89888), RIPA buffer (cat no. J63306.AP), Lipofectamine™ 3000 Transfection Reagent (cat no. 13778150), ViraPower Lentiviral Packaging Mix (cat no. K497500), Protease Inhibitor Cocktail I (cat no. J64401.LQ), Pierce Direct Magnetic IP/Co-IP Kit (cat no. 88828), protein-G Dynabeads (cat no. 10003D), NP-40 lysis buffer (cat no. J60766.AP) were purchased from Thermal Scientific; OPTI-MEM reduced serum medium (cat no.31985-062) was from Gibco, USA;

Antibodies against β-catenin (cat no. ab223075, 1:1000 dilution), HER2 (cat no. ab134182, 1:1000 dilution), HER2 (phospho Y1248) (cat no. ab201013, 1:1200 dilution), HER3 (cat no. ab32121, 1:1000 dilution), HER3 (phospho Y1289) (cat no. ab76469, 1:1000 dilution), SRC (cat no. ab133283 1:1000 dilution), SRC (phospho Y416) (cat no. ab278693, 1:1000 dilution), horseradish peroxidase (HRP)-conjugated secondary antibody (cat no. ab6728, 1:1500), goat anti-mouse IgG H&L (Alexa Fluor® 488) (cat no. ab150113, 1:1500), goat anti-mouse IgG H&L (Alexa Fluor® 488) (cat no. ab150113, 1:1500), goat anti-mouse IgG H&L (Alexa Fluor® 555) (cat no. ab150114, 1:1500) ECL kit (cat no. ab133406) were purchased from Abcam, Waltham, MA, USA. FLAG antibody (cat no. F1804, 1:1500), type VII agarose (cat no. 39346-81-1) was purchased from Sigma-Aldrich. Colony counter (PhenoBooth+) was purchased from Singer Instruments, UK. Trans-Blot Turbo Transfer System was a product of Bio-Rad laboratories, USA.

Immunohistochemistry

The 5 μm transverse tissue sections were deparaffinized and rehydrated by going through an ethanol gradient. After washing in water, the slides were autoclaved for 4 min in sodium citrate buffer for antigen retrieval. Endogenous peroxidase activity was suppressed by treating with hydrogen peroxidase for 5 min at room temperature. The tissue sections were rinsed with tris buffered saline 1X (TBS), reacted to primary antibody against β-catenin for 1 h and subsequently washed with TBS for three times, and incubated with horse radish peroxidase-conjugated anti-goat IgG (H+L) for 30 min. Diaminobenzidine (DAB) and haematoxylin chromogen (Dako, Glostrup, Denmark) method was used to visualize the immunoreactivity. The slides were examined under a light microscope to score β-catenin-positive cells. For each sample, 10 randomly selected fields were evaluated.

Cell lines and culture conditions

Human cells MCF7 (cat no. HTB-22), BT474 (cat no. HTB-20), SKBr3 (cat no. HTB-30), and MDA-MB-231 (cat no. HTB-26) were purchased from American Type Collection Center, Manassas, VA, USA and were cultured in Eagle's minimum essential medium (EMEM)

(ATCC cat no. 20-2003) with 10% fetal bovine serum (FBS, (ATCC cat no. 30-2020), and 50 U/ml penicillin, and 50 μg/ml streptomycin sulfate (Fisher Scientific MT30001CI). All cell lines were cultured in 5% CO_2 at 37°C in a humidified incubator as instructed by the manufacturer. HEK293T cells were grown to produce virus for transfection experiment as previously described (Cipriano *et al*., 2010) in DMEM medium (cat no. 12491, Thermo Scientific, Wilmington, Delaware, USA) supplemented with 5% FBS. Cultured cells were routinely tested for mycoplasma contamination using MycoSEQ Mycoplasma Detection Kit (cat no. 4460626, Thermo Scientific) to ensure they were mycoplasma-free.

Vectors and cell transfection

To generate the cell lines overexpressing β-catenin and HER2, Lentiviral vectors pLV-Puro-CMV containing β-catenin and HER2 were constructed (Cyagen Biosciences, USA). BT474 and SKBr3 cells were seeded into the wells of 12-well plates at a density of 1×10^5 cells/well, grown to a confluency of 70–80%. The cells were transfected with vectors at multiplicity of infection (MOI) 50 based on the results of a pilot study. Mock transfection with LV-EGFP was employed as a negative control. Cell morphology was examined after transfection and the culture medium was refreshed 12 h after the transfection. Stably transfected clones were selected using puromycin at 1.5 μg/ml. The expression of genes was assessed using Western blot analysis.

Plasmids encoding shRNAs targeting SRC were designed using tools provided by the Genetic Perturbation Platform (https://portals.broadinstitute.org/gpp/public/) and inserted into pLKO.1. Lentiviral vectors were transfected into HEK293T cells using Lipofectamine 2000 together with ViraPower Lentiviral Packaging Mix according to the manufacturer's instructions. Supernatants containing viruses were collected and used to infect cells for 16 hours. Cells at 90% confluency were harvested, re-suspended in FBS-free DMEM medium, transfected with the shRNA vectors (5 μg/well) and 250 μl Opti-MEM and Lipofectamine 3000 according to the manufacturer's instructions. After incubation for 20 min, the transfection mixtures were added to the wells of 24-well plates to culture for 4 h.

Immunoblot and immunoprecipitation

Cells were harvested by centrifugation at 500×*g* at room temperature, lysed using RIPA buffer containing protease inhibitor cocktail. The protein content was determined using BCA protein assay kit according to the manufacturer's instructions. About 50 μg protein per lane was loaded for 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or used for an immunoprecipitation reaction. For immunoprecipitation, 5 μg FLAG antibody was mixed with 50 μl PBS-washed protein-G Dynabeads in 500 μl RIPA lysis buffer containing protease and phosphatase inhibitors by rotating for 2 hours at 4°C. The complex beads were washed twice with PBS and added with protein lysates. The antibody-bead conjugates were rotated at 4°C for 2 hours, washed eight times with NP40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris-Cl pH 8.0) and resuspended in 25 μl of 2X SDS BME loading dye for immunoblot analysis. After separation by SDS-PAGE, the proteins were transferred electrically to polyvinylidene difluoride (PVDF) membranes ((Millipore)) using Trans-Blot Turbo Transfer System. The membranes were blocked with 5% non-fat dry milk in 1X TBS buffer containing 0.1% Tween 20 for 6 h at 25°C and incubated with proper primary antibodies at 4°C overnight. After being rinsed three times with the same buffer, the blots were reacted with HRP-conjugated secondary antibodies at 25°C for 2 h and then visualized using the ECL kit as instructed by the supplier. Densitometric determination was conducted using Quantity One software (version v4.6.1; Bio-Rad Laboratories, Inc.) using β-actin as the internal control.

Xenograft assays

Eight-week-old specific-pathogen free (SPF) BALB/c-nu nude mice (female, weight 25–30 g) purchased from Saiye Biotech, Shanghai, were used. The mice were housed in hygienic and pathogen-free conditions at 25–26°C with good ventilation. They had free access to filtered water and diet under a 12/12 hours day/night cycle. Mice were randomly divided (n=36) and MCF7 cells expressing β-catenin and HER2 (100 μl, 5×106) were orthotopically injected into the mammary glands for BC development. Animals bearing tumors were then randomized into cohorts to receive administration of trastuzumab, which was administered intraperitoneally for two weeks (6 times) with a total dose of 10 mg/kg. (n=6 in each group). The control group received PBS only. Mouse sizes were recorded every five days till 35 days after injection. Gross necropsies were performed, and tissues were collected and snap frozen in liquid nitrogen for downstream analysis. At different times after the implanting, mice were sacrificed by euthanization with carbon dioxide applied at a flow rate of 20% of the cage volume per minute (5 L/min) and tumors were isolated for size measurement using Vernier caliper.

The animal experimental protocols were approved by Animal Research Ethics Committee of the Shenzhen Hospital Affiliated with Southern Medical University.

Soft agar assay

Soft agar assays were performed to assess the colony formation ability as previously described (Horibata *et al*., 2015). Briefly, MCF7 cells (1×10^5 cells/ml) were suspended gently in 0.6% type VII agarose (maintained at 42°C) prepared in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were plated in triplicate onto a bottom layer of 1.2% agar in 60-mm dishes. After being solidified at room temperature for 30 min, the plates were covered and were placed into a 37°C humidified incubator to culture. Three weeks later, the cells were stained with 200 μl of 0.1% crystal violet per well overnight at 37°C. The stained colonies were analyzed using PhenoBooth+colony counter.

Confocal laser immunofluorescence assay

Human breast adenocarcinoma MDA-MB-231 cells were air dried on siliconized glass slides (Dako, Kyoto, Japan) at room temperature, fixed in pre-chilled methanol for 2 min and washed three times with PBS. The cells on the slides were incubated with 100 μl of each antibody against β-catenin and HER2 overnight at 4°C in a humid box. After being washed with PBS, the slides were incubated with 100 μl of goat anti-mouse IgG H&L (Alexa Fluor® 488) or goat anti-mouse IgG H&L (Alexa Fluor® 555) for 30 min. The slides were washed three times with PBS and countered stained with DAPI and mounted with buffered glycerol. The cells were visualized and photographed using a Leica confocal laser scan-

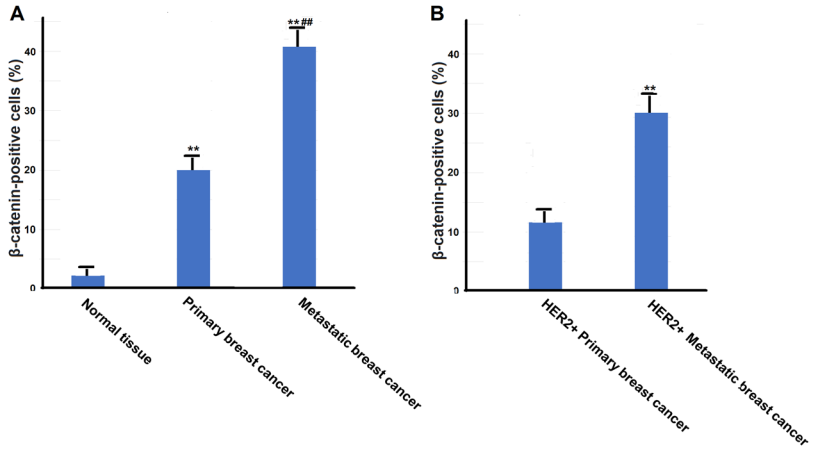

Figure 1. Percentage of β-catenin-positive cells in BC and HER2+BC.
(**A**) in normal (n=55), primary BC (n=45) and metastatic BC (n=39). ** and ## denote $P<0.01$ *vs* normal and primary BC tissues, respectively. (**B**) in HER2+ primary BC and HER2 metastatic BC. ** denotes $P<0.01$ *vs* primary BC tissues, respectively.

ning microscope equipped with an OG 515 filter (Leica AG, Heerbrugg, Switzerland).

Co-immunoprecipitation

Co-immunoprecipitation of β-catenin and HER2 was performed using Direct Magnetic IP/Co-IP Kit according to supplier's protocols. Briefly, confluent MDA-MB-231 cells expressing β-catenin and HER2 were added with ice-cold IP lysis buffer and the lysates were centrifuged at ~13 00×*g* for 10 minutes to pellet the cell debris. The supernatant was added to anti-HER2 antibody coupled beads and gently mixed and incubated on a rotating platform for 30–60 minutes at room temperature. After being washed with a washing buffer, the beads were collected with a magnetic stand and the proteins were eluted and analyzed using Western blot analysis.

Statistical analysis

Statistical analyses were performed with SPSS (version 19.0; SPSS, Inc., Chicago, IL, USA). Data are expressed as the means ± S.D. Differences between multiple sample means were analyzed using one-way ANOVA and the LSD-t method was used for comparison between the two groups. Statistical significance was defined as a two-tailed $P<0.05$.

RESULTS

β-catenin is highly expressed in primary and metastatic BC

The Wnt/β-catenin signaling pathway is well known to be involved in various human cancers. To demonstrate the role of β-catenin in human BC, we first analyzed the expression of β-catenin in normal tissues and BC tissues in the clinical samples using immunohistochemistry methods. It was found that β-catenin was expressed in 40.8% cells in the metastatic BC tissues, 20.1% cells in primary BC tissues and cells, and 2.2% in non-cancer tissues (Fig. 1A). Furthermore, among HER2+ BC cells, β-catenin was overexpressed in 12% of primary BC cells and 30% of metastatic BC cells (Fig. 1B).

β-catenin and HER2 synergically promote tumor development

To assess the synergy of β-catenin and HER2 in driving tumorigenesis, we established MCF7 cells that stably expressed β-catenin and HER2. Western blot analysis showed that non-transfected MCF7 cells (control) had very low background β-catenin and HER2 expression, and in the transformed cells β-catenin and HER2 expressions were significantly upregulated ($P<0.01$, Fig. 2A). Notably, only cells expressing both β-catenin and HER2 exhibited an unusual and invasive phenotype when cultured in monolayers, while single expression of either β-catenin or HER2 did not change cellular morphology dramatically (Fig. 2B). To assess the malignancy of transformed cells, soft-agar experiments were performed. Cells expressing β-catenin and HER2 alone formed more colonies than control and had similar number of colonies. However, the number of colonies formed by cells expressing both β-catenin and HER2 was more than doubled as compared with cells expressing single genes ($P<0.01$, Fig. 2C). To further confirm these findings, MCF7 cells expressing β-catenin and HER2 were used to inoculate immunodeficient mice for tumor development. The animal experiments demonstrated that overexpression of both HER2 and β-catenin significantly promoted tumor formation and progression, resulting in significantly large tumors, although β-catenin and HER2 alone induced tumor formation ($P<0.01$, Fig. 2D), suggesting that β-catenin might synergize with HER2 to promote the formation and development of tumor cells.

β-catenin promotes the phosphorylation of HER and tumorigenesis

To explore the effect of β-catenin on HER phosphorylation, we overexpressed β-catenin in two HER2-elevated BC cell lines BT474 and SKBr3. We used retrovirus containing GFP-β-catenin or GFP (control) to overexpress β-catenin in these two cell lines. The results showed that compared with the control, β-catenin was remarkably up-regulated. Meanwhile, phosphorylation of HER2 at Y1248 and HER3 at Y1289 was significantly increased ($P<0.01$, Fig. 3A). Notably, the phosphorylation levels of HER2 and HER3 were also significantly increased when cells were serum-starved (Fig. 3A).

To further examine the effect of β-catenin overexpression on the progression of HER2+ BC, we orthotopi-

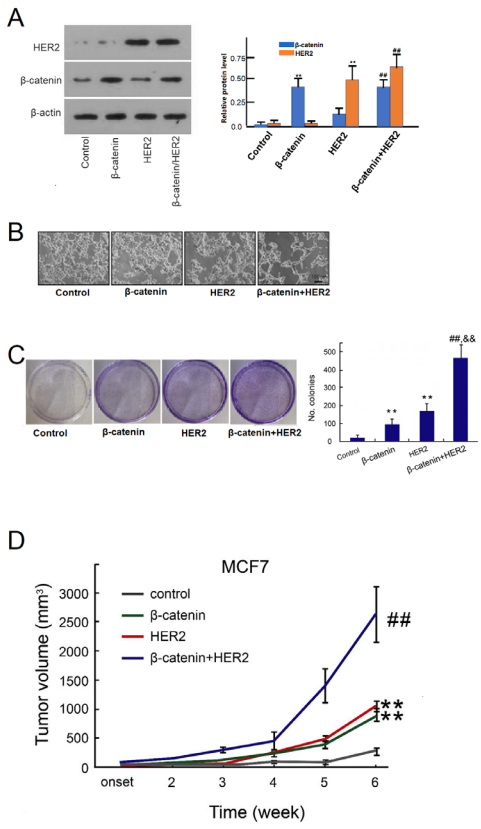

Figure 2. β-catenin and HER2 expression (A), morphology change (B), colony formation (C) and tumor development (D) by stably transformed MCF7 cells.
(**A**) left panel: Western blots, right panel: relative protein level, ** and ## denote $P<0.01$ *vs* control and HER2, respectively. (**B**) invasive morphology. (**C**) left panel: colony formation by soft agar assay, right panel: colony number, **, ## and && denote $P<0.01$ *vs* control, β-catenin and HER2, respectively. (**D**) tumor development in nut mice (n=6). ** and ## denote $P<0.01$ *vs* control, β-catenin and HER2, respectively.

cally injected BT474 cells expressing β-catenin into the mouse mammary glands in immunodeficient mice, and measured tumor growth. The results showed that compared with the control group, at 25 days post-injection, the tumors formed with β-catenin overexpressing cells were significantly greater than control ($P<0.01$, Fig. 3B).

β-catenin binds to HER2

Since β-catenin can promote the phosphorylation of HER2, we set to investigate if β-catenin and HER2 are physically in contact with each other. Using confocal immunofluorescence assay, we found that β-catenin and HER2 were co-localized on the cell membrane in MDA-MB-231 cell line (Fig. 4A), suggesting that they might be in direct contact with each other. We further performed co-immunoprecipitation of the proteins with extracts from SKBr3 and BT4742 cell lines. It was found that β-catenin and HER2 were co-precipitated in both cell lines (Fig. 4B).

β-catenin enhances HER2 interaction with SRC

Elevated SRC has been shown to stabilize HER2 to enhance cellular migration and survival (Mayer & Krop, 2010). Therefore, we investigated the impact of β-catenin on SRC and its phosphorylation. When β-catenin was knockdown in MDA-MB-361 cells, SRC and its phosphorylation, and HER2 phosphorylation at Y877and

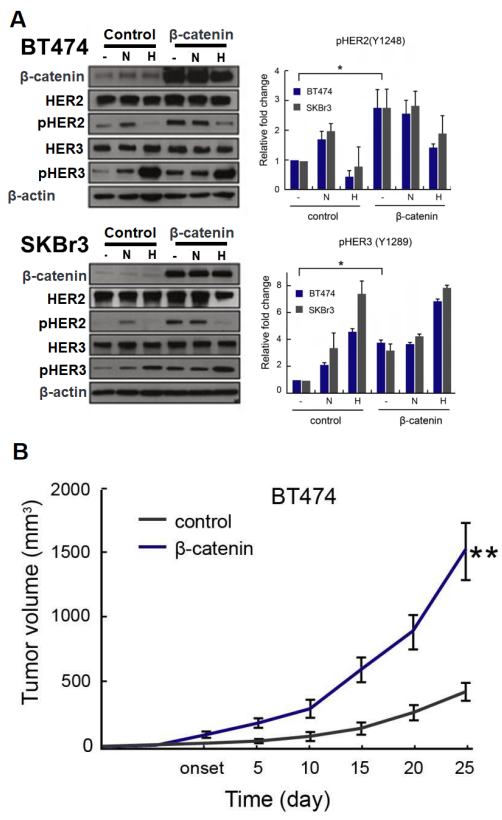

Figure 3. Phosphorylation of HER2 and HER3 expression by β-catenin in HER2-elevated BC cells BT474 and SkBr3c and tumor formation by BT474 overexpressing β-catenin in immunodeficient mice (n=6).
(**A**) Left panel: representative Western blots, right panel: relative protein level. (**B**) tumor size in nut mice. ** denote $P<0.01$ *vs* control.

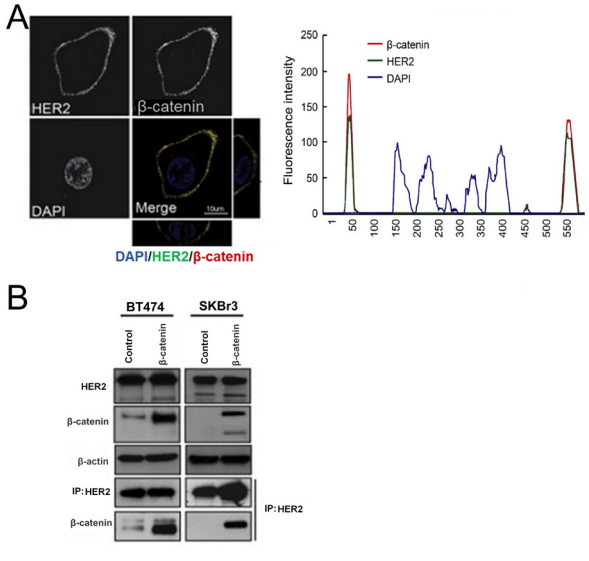

Figure 4. Cellular localization and immunoprecipitation of β-catenin and HER2.
(**A**) left panel: confocal immunofluorescence microscopy, (**B**) fluorescence intensity. (**B**) immunoprecipitation assays of β-catenin and HER2 using SKBr3 and BT474 cell extracts.

Y1248 were significantly reduced (Fig. 4A). To explore whether the inactivation of SRC would affect the activation of HER by β-catenin, SRC was downregulated using two SRC shRNAs in SKBr3 cells expressing β-catenin.

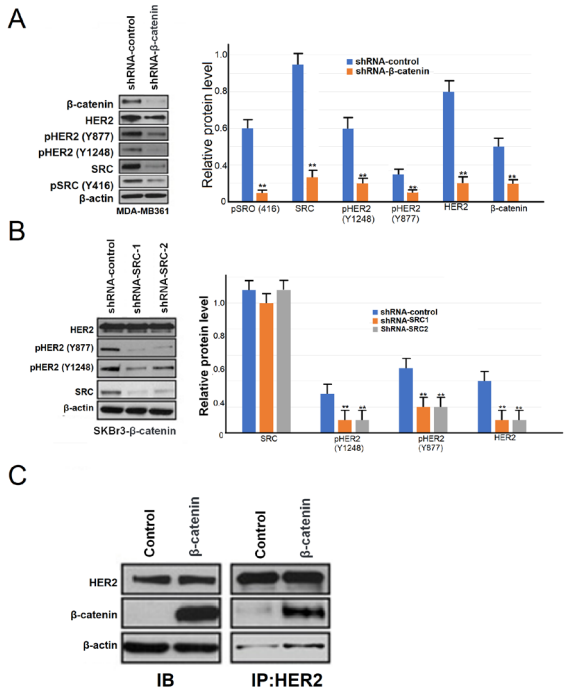

Figure 5. Phosphorylation of SRC and HER2 after knockdown of β-catenin (A) and SRC (B) and interaction between SRC and HER2 after overexpressing β-catenin (C).
Left panel: representative Western blots, right panel: relative protein level. ** denote $P<0.01$ *vs* control.

As a result, we found that HER2 phosphorylation at Y877 and Y1248 were decreased in the SRC-knockdown cells (Fig. 5B). These results suggest that SRC might regulate β-catenin-mediated HER2 phosphorylation to impact tumor development. On the other hand, when β-catenin was overexpressed, the interaction between HER2 and SRC was enhanced (Fig. 5C).

To further elucidate the functional relevance among β-catenin, HER2, and SRC, we examined the effect of β-catenin overexpression on drug sensitivity of the tumor cells. Recent studies have shown that abnormal activation of SRC can lead to resistance of tumor cells to trastuzumab. We found that in HER2$^+$ BC, overexpression of β-catenin increased the resistance to trastuzumab (Fig. 6A). Further analysis found that trastuzumab inhibited the phosphorylation of HER2, but the SRC remained highly active in cells overexpressing β-catenin as compared to control cells (Fig. 6B).

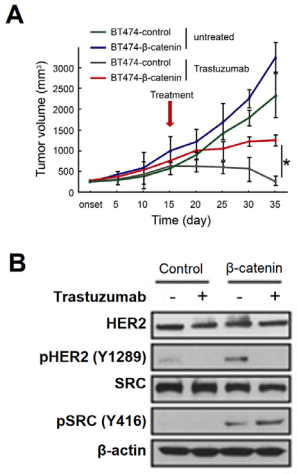

Figure 6. Development of tumors after trastuzumab treatment (A) and phosphorylation of SRC (B) in mice (n=6) xenografted with β-expressing BT474 cells.
*denotes $P<0.01$ *vs* control.

DISCUSSION

A large number of *in vitro* and *in vivo* studies have shown that HER2 overexpression is related to the occurrence of BC (Loibl & Gianni, 2017). However, there is not enough evidence to confirm that HER2 can drive the transformation from benign to malignant and metastatic BC (Exman & Tolaney, 2021). It is also not clear whether other genomic variations can synergically promote BC with HER2. In this study, we found that β-catenin appears to be an important regulatory protein of HER2 that drives BC development and resistance to trastuzumab. β-catenin is highly expressed in BC, particularly in metastatic BC, and synergically promotes the development of BC in xenograft mouse models probably by increasing the phosphorylation of HER2. We also found that β-catenin binds with HER2 and enhances the interaction between HER2 and SRC, resulting in increased trastuzumab resistance. The findings from this study provide new insights and clues for the development of new therapeutic strategies and targets for BC.

Better understanding the molecular mechanisms underlying the metastatic dissemination of HER2+BC would be helpful to the development of effective clinical interventions. Wnt/β-catenin signaling pathway is well known to play important roles in human cancers (Zhang & Wang, 2020). Assessment of β-catenin expression in tumor tissues has shown that it is upregulated in BC, particularly in metastatic cancer. As a multifunctional protein with a central role in physiological homeostasis, β-catenin is often upregulated in various diseases, including cancer, as the result of mutations of canonical Wnt signaling pathway genes (Cui *et al.*, 2018; White *et al.*, 2012). In canonical Wnt pathway, Dsh, β-catenin, glycogen synthase kinase 3 beta (GSK3β), adenomatous polyposis coli (APC), AXIN, and T-cell factor (TCF)/lymphoid enhancement factor (LEF) are the major signal transducers with β-catenin as core protein (Behrens *et al.*, 1996; Yost *et al.*, 1996). Our study further demonstrated that β-catenin is highly upregulated in HER2 BC, suggesting that there might be interactions between the two genes, particularly in metastatic BC. HER2 has been shown to interact with oncogenic/stemness signaling pathways including Wnt/β-catenin, and HER2 overexpression promotes EMT and the emergence of cancer stem cell properties in BC (Nami & Wang, 2017). Using MCF7 cells, we found that although β-catenin and HER2 alone increase the colony formation ability, coexpression of the two genes results in increased invasive transformation of the cells and higher tumorigenicity in immunodeficient mice as compared to single β-catenin or HER2 protein. These studies further demonstrate that β-catenin and HER2 have synergy in promoting BC. In gastric cancer cells, overexpression of HER2 was shown to increase the stemness and invasiveness, which is regulated by Wnt/β-catenin signaling (Jung *et al.*, 2019). Previously the aberrant expression of β-catenin was associated with more aggressive behaviors of BC although it was not statistically correlated to HER2 (Sefidbakht *et al.*, 2021). However, in epithelial ovarian cancer (EOC), high expression of β-catenin was found in HER2-positive EOC tissue samples and was correlated with a poor

patient prognosis. Therefore, Wnt3a/β-catenin/TCF7L2 signaling axis is considered to play an important role in EOC resistance to trastuzumab and HER2-targeted drugs may have the potential for EOC treatment (Shen *et al.*, 2020).

To elucidate the interaction between β-catenin and HER2, we investigated the effect of β-catenin on the HER activity by overexpressing β-catenin in BC cell lines BT474 and SKBr3 with elevated HER2 level. It was found that in these cells, there was increased phosphorylation of HER2 and HER3. Previously, HER2 phosphorylation was found to be due to the activation of other HER receptors, such as reactivation of HER3 and ADAM17-mediated ligand release (Gijsen *et al.*, 2010). In addition, the levels of phosphorylated HER2 increased significantly in cells treated with arenite and exogenous EGF, TGFα, NRG1, and HSP90 through the dimerization of HER2 with other members of HER family, such as HER3 (Jin *et al.*, 2018). Other proteins reported to phosphorylate HER2 include PTPN18 that regulates HER2-mediated cellular functions through defining both its phosphorylation and ubiquitination barcodes (Wang *et al.*, 2014). The activation (phosphorylation) of HER family gene by β-catenin is likely partially responsible for the enhanced tumorigenesis. Study with β-catenin knockdown may further define the relationship between β-catenin and HER2 activation.

To further investigate the model of action of β-catenin and HER2, we studied the co-location of these two proteins using confocal immunofluorescence assay. β-catenin and HER2 were found to be co-localized on the cell membrane, indicating that they might have direct physical contact with each other, and the results from immunoprecipitation in the two cell lines tested support this speculation. To further refine the interaction between β-catenin and HER2, protein variants might be used to better understand the structure-binding relationship.

SRC is one of the non-receptor tyrosine kinases that participates in cell proliferation, differentiation, survival, and invasion through the PI3K, MAPK, STAT3, FAK signaling pathways (Roskoski Jr., 2015). Studies have shown that increased SRC activation can stabilize HER2 to enhances cellular migration and survival and confers resistance of $HER2^+$ BC cells to trastuzumab (Peiro *et al.*, 2014). Our analysis showed that the activation (phosphorylation) of both SRC and HER2 is reduced once β-catenin is knockdown, implying that β-catenin might be involved in regulating both SRC and HER2 signaling. On the other hand, knockdown of SRC in SKBr3 cells expressing β-catenin results in decreased HER2 phosphorylation, suggesting that SRC might regulate β-catenin-mediated HER2 phosphorylation to impact tumor development, and this SRC interaction with HER2 is enhanced by β-catenin. Since increased trastuzumab resistance and SRC activation were observed in tumors derived from cells expressing β-catenin, it is likely that SRC might also play a role in the drug resistance. Trastuzumab is a monoclonal antibody that binds HER2 extracellular subdomain IV to treat BC, although its mechanism of action is still not fully understood (Shawver *et al.*, 2002). It was demonstrated to reduce HER2 phosphorylation (Nami *et al.*, 2019). This is consistent with our results that following trastuzumab treatment, phosphorylation of HER2 is reduced. On the other hand, the activation-associated phosphorylation of SRC at tyrosine 416 remained significantly higher in tumors derived from cells expressing β-catenin than control, irrespective of trastuzumab treatment, suggesting that SRC activation is involved in trastuzumab resistance. Previously, the SRC/STAT3 signaling pathway was found to be activated by chromosome condensin 1 complex subunit G (NCAPG), leading to trastuzumab resistance in $HER2^+$BC and SRC phosphorylation may enhance nuclear localization and activation of STAT3 (Jiang *et al.*, 2020), leading to trastuzumab resistance (Zhang *et al.*, 2011). Since SRC phosphorylation occurs in tumors derived from cells overexpression β-catenin, further study is needed to explore β-catenin as a therapeutic target to overcome trastuzumab resistance.

CONCLUSION

β-catenin is highly expressed in BC tissues as compared to non-cancer tissue. It synergically promotes formation and development of BC with HER2. β-catenin interacts with HER2 leading to SRC activation and trastuzumab resistance. Due to these important roles, β-catenin may be further explored as a therapeutic target to overcome trastuzumab resistance in BC.

Declarations

Ethics approval and consent to participate: the Ethics Committee of the Shenzhen Hospital Affiliated with Southern Medical University and written informed consent was obtained from every participant.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: None.

Funding: None.

Acknowledge: Not applicable.

REFERENCES

Anastasiadi Z, Lianos GD, Ignatiadou E, Harissis HV, Mitsis M (2017) Breast cancer in young women: an overview. *Updates Surg* **69**: 313–317. https://doi.org/10.1007/s13304-017-0424-1

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W (1996) Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **382**: 638–642. https://doi.org/10.1038/382638a0

Bredin P, Walshe JM, Denduluri N (2020) Systemic therapy for metastatic HER2-positive breast cancer. *Semin Oncol* **47**: 259–269. https://doi.org/10.1053/j.seminoncol.2020.07.008

Brennan KR, Brown AM (2004) Wnt proteins in mammary development and cancer. *J Mammary Gland Biol Neoplasia* **9**: 119–131. https://doi.org/10.1023/B:JOMG.0000037157.94207.33

Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro Jr. G, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, McFadden E, Leyland-Jones B, Bell R, Dowsett M, Jackisch C, Herceptin Adjuvant Trial Study T (2017a) 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet* **389**: 1195–1205. https://doi.org/10.1016/S0140-6736(16)32616-2

Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro Jr. G, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, McFadden E, Leyland-Jones B, Bell R, Dowsett M, Jackisch C, Herceptin Adjuvant Trial Study T (2017b) 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet* **389**: 1195–1205. https://doi.org/10.1016/S0140-6736(16)32616-2

Cipriano R, Patton JT, Mayo LD, Jackson MW (2010) Inactivation of p53 signaling by p73 or PTEN ablation results in a transformed phenotype that remains susceptible to Nutlin-3 mediated apoptosis. *Cell Cycle* **9**: 1373–1379. https://doi.org/10.4161/cc.9.7.11193

Cui C, Zhou X, Zhang W, Qu Y, Ke X (2018) Is beta-Catenin a Druggable Target for Cancer Therapy? *Trends Biochem Sci* **43**: 623–634. https://doi.org/10.1016/j.tibs.2018.06.003

Dankort D, Maslikowski B, Warner N, Kanno N, Kim H, Wang Z, Moran MF, Oshima RG, Cardiff RD, Muller WJ (2001) Grb2 and Shc adapter proteins play distinct roles in Neu (ErbB-2)-induced mammary tumorigenesis: implications for human breast cancer. *Mol*

Cell Biol **21**: 1540–1551. https://doi.org/10.1128/MCB.21.5.1540-1551.2001
Derakhshani A, Rezaei Z, Safarpour H, Sabri M, Mir A, Sanati MA, Vahidian F, Gholamiyan Moghadam A, Aghadoukht A, Hajiasgharzadeh K, Baradaran B (2020) Overcoming trastuzumab resistance in HER2-positive breast cancer using combination therapy. *J Cell Physiol* **235**: 3142–3156. https://doi.org/10.1002/jcp.29216
Doumpas N, Lampart F, Robinson MD, Lentini A, Nestor CE, Cantu C, Basler K (2019) TCF/LEF dependent and independent transcriptional regulation of Wnt/beta-catenin target genes. *EMBO J* **38**: https://doi.org/10.15252/embj.201798873
Ellis H, Ma CX (2019) PI3K Inhibitors in Breast Cancer Therapy. *Curr Oncol Rep* **21**: 110. https://doi.org/10.1007/s11912-019-0846-7
Escriva-de-Romani S, Arumi M, Bellet M, Saura C (2018) HER2-positive breast cancer: Current and new therapeutic strategies. *Breast* **39**: 80–88. https://doi.org/10.1016/j.breast.2018.03.006
Exman P, Tolaney SM (2021) HER2-positive metastatic breast cancer: a comprehensive review. *Clin Adv Hematol Oncol* **19**: 40–50.
Fahad Ullah M (2019) Breast Cancer: Current Perspectives on the Disease Status. *Adv Exp Med Biol* **1152**: 51–64. https://doi.org/10.1007/978-3-030-20301-6_4
Ganz PA, Goodwin PJ (2015) Breast Cancer Survivorship: Where Are We Today? *Adv Exp Med Biol* **862**: 1–8. https://doi.org/10.1007/978-3-319-16366-6_1
Gijsen M, King P, Perera T, Parker PJ, Harris AL, Larijani B, Kong A (2010) HER2 phosphorylation is maintained by a PKB negative feedback loop in response to anti-HER2 herceptin in breast cancer. *PLoS Biol* **8**: e1000563. https://doi.org/10.1371/journal.pbio.1000563
Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, Ruddy K, Tsang J, Cardoso F (2019) Breast cancer. *Nat Rev Dis Primers* **5**: 66. https://doi.org/10.1038/s41572-019-0111-2
Horibata S, Vo T v, Subramanian V, Thompson PR, Coonrod SA (2015) Utilization of the soft agar colony formation assay to identify inhibitors of tumorigenicity in breast cancer cells. *J Vis Exp* e52727. https://doi.org/10.3791/52727
Hsieh TH, Hsu CY, Tsai CF, Chiu CC, Liang SS, Wang TN, Kuo PL, Long CY, Tsai EM (2016) A novel cell-penetrating peptide suppresses breast tumorigenesis by inhibiting beta-catenin/LEF-1 signaling. *Sci Rep* **6**: 19156. https://doi.org/10.1038/srep19156
Hunter FW, Barker HR, Lipert B, Rothe F, Gebhart G, Piccart-Gebhart MJ, Sotiriou C, Jamieson SMF (2020) Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. *Br J Cancer* **122**: 603–612. https://doi.org/10.1038/s41416-019-0635-y
Jiang L, Ren L, Chen H, Pan J, Zhang Z, Kuang X, Chen X, Bao W, Lin C, Zhou Z, Huang D, Yang J, Huang H, Wang L, Hou N, Song L (2020) NCAPG confers trastuzumab resistance via activating SRC/STAT3 signaling pathway in HER2-positive breast cancer. *Cell Death Dis* **11**: 547. https://doi.org/10.1038/s41419-020-02753-x
Jin P, Liu J, Wang X, Yang L, Zhou Q, Lin X, Xi S (2018) HER2 Activation factors in arsenite-exposed bladder epithelial cells. *Toxicol Sci* **166**: 354–369. https://doi.org/10.1093/toxsci/kfy202
Jung DH, Bae YJ, Kim JH, Shin YK, Jeung HC (2019) HER2 Regulates cancer stem cell activities *via* the Wnt signaling pathway in gastric cancer cells. *Oncology* **97**: 311–318. https://doi.org/10.1159/000502845
Kolak A, Kaminska M, Sygit K, Budny A, Surdyka D, Kukielka-Budny B, Burdan F (2017) Primary and secondary prevention of breast cancer. *Ann Agric Environ Med* **24**: 549–553. https://doi.org/10.26444/aaem/75943
Larionov AA (2018) Current therapies for human epidermal growth factor receptor 2-positive metastatic breast cancer patients. *Front Oncol* **8**: 89. https://doi.org/10.3389/fonc.2018.00089
Lee YY, Kim HP, Kang MJ, Cho BK, Han SW, Kim TY, Yi EC (2013) Phosphoproteomic analysis identifies activated MET-axis PI3K/AKT and MAPK/ERK in lapatinib-resistant cancer cell line. *Exp Mol Med* **45**: e64. https://doi.org/10.1038/emm.2013.115
Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, Blattler WA, Lambert JM, Chari R v, Lutz RJ, Wong WL, Jacobson FS, Koeppen H, Schwall RH, Kenkare-Mitra SR, Spencer SD, Sliwkowski MX (2008) Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* **68**: 9280–9290. https://doi.org/10.1158/0008-5472.CAN-08-1776
Liu H, Mastriani E, Yan ZQ, Yin SY, Zeng Z, Wang H, Li QH, Liu HY, Wang X, Bao HX, Zhou YJ, Kou JJ, Li D, Li T, Liu J, Liu Y, Yin L, Qiu L, Gong L, Liu SL (2016) SOX7 co-regulates Wnt/beta-catenin signaling with Axin-2: both expressed at low levels in breast cancer. *Sci Rep* **6**: 26136. https://doi.org/10.1038/srep26136
Loibl S, Gianni L (2017) HER2-positive breast cancer. *Lancet* **389**: 2415–2429. https://doi.org/10.1016/S0140-6736(16)32417-5
Mayer EL, Krop IE (2010) Advances in targeting SRC in the treatment of breast cancer and other solid malignancies. *Clin Cancer Res* **16**: 3526–3532. https://doi.org/10.1158/1078-0432.CCR-09-1834
Mir MA, Qayoom H, Mehraj U, Nisar S, Bhat B, Wani NA (2020) Targeting different pathways using novel combination therapy in triple negative breast cancer. *Curr Cancer Drug Targets* **20**: 586–602. https://doi.org/10.2174/1570163817666200518081955
Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ (2006) Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract Oncol* **3**: 269–280. https://doi.org/10.1038/ncponc0509
Nami B, Wang Z (2017) HER2 in Breast cancer stemness: a negative feedback loop towards trastuzumab resistance. *Cancers (Basel)* **9**: https://doi.org/10.3390/cancers9050040
Nami B, Maadi H, Wang Z (2019) The effects of pertuzumab and its combination with Trastuzumab on HER2 homodimerization and phosphorylation. *Cancers (Basel)* **11**: https://doi.org/10.3390/cancers11030375
Peairs KS, Choi Y, Stewart RW, Sateia HF (2017) Screening for breast cancer. *Semin Oncol* **44**: 60–72. https://doi.org/10.1053/j.seminoncol.2017.02.004
Peiro G, Ortiz-Martinez F, Gallardo A, Perez-Balaguer A, Sanchez-Paya J, Ponce JJ, Tibau A, Lopez-Vilaro L, Escuin D, Adrover E, Barnadas A, Lerma E (2014) Src, a potential target for overcoming trastuzumab resistance in HER2-positive breast carcinoma. *Br J Cancer* **111**: 689–695. https://doi.org/10.1038/bjc.2014.327
Rajarajan N, Mariotti V, Basu A, Kodumudi K, Han H, Czerniecki B, Hoover S (2020) Strategies to Combat Human Epidermal Growth Factor Receptor 2 (HER2) Resistance in HER2-positive breast cancer. *Crit Rev Oncog* **25**: 209–231. https://doi.org/10.1615/CritRevOncog.2020036417
Ramagopalan S v, Pisoni R, Zenin A, Rathore LS, Ray J, Sammon C (2021) Comparative effectiveness of trastuzumab emtansine versus lapatinib plus chemotherapy for HER2+ metastatic breast cancer. *J Comp Eff Res* **10**: 595–602. https://doi.org/10.2217/cer-2020-0201
Rinnerthaler G, Gampenrieder SP, Greil R (2019) HER2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer. *Int J Mol Sci* **20**: https://doi.org/10.3390/ijms20051115
Roskoski Jr. R (2015) Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. *Pharmacol Res* **94**: 9–25. https://doi.org/10.1016/j.phrs.2015.01.003
Ruiz-Saenz A, Dreyer C, Campbell MR, Steri V, Gulizia N, Moasser MM (2018) HER2 Amplification in tumors activates PI3K/Akt signaling independent of HER3. *Cancer Res* **78**: 3645–3658. https://doi.org/10.1158/0008-5472.CAN-18-0430
Santa-Maria CA, Nye L, Mutonga MB, Jain S, Gradishar WJ (2016) Management of metastatic HER2-positive breast cancer: where are we and where do we go from here? *Oncology (Williston Park)* **30**: 148–155
Saura C, Oliveira M, Feng YH, Dai MS, Chen SW, Hurvitz SA, Kim SB, Moy B, Delaloge S, Gradishar W, Masuda N, Palacova M, Trudeau ME, Mattson J, Yap YS, Hou MF, De Laurentiis M, Yeh YM, Chang HT, Yau T, Wildiers H, Haley B, Fagnani D, Lu YS, Crown J, Lin J, Takahashi M, Takano T, Yamaguchi M, Fujii T, Yao B, Bebchuk J, Keyvanjah K, Bryce R, Brufsky A (2020) Neratinib plus capecitabine *versus* Lapatinib plus capecitabine in HER2-positive metastatic breast cancer previously treated with >/= 2 her2-directed regimens: phase III NALA trial. *J Clin Oncol* **38**: 3138–3149. https://doi.org/10.1200/JCO.20.00147
Schade B, Lesurf R, Sanguin-Gendreau V, Bui T, Deblois G, O'Toole SA, Millar EK, Zardawi SJ, Lopez-Knowles E, Sutherland RL, Giguere V, Kahn M, Hallett M, Muller WJ (2013) beta-Catenin signaling is a critical event in ErbB2-mediated mammary tumor progression. *Cancer Res* **73**: 4474–4487. https://doi.org/10.1158/0008-5472.CAN-12-3925
Sefidbakht S, Saeedipour H, Saffar H, Mirzaian E (2021) Determination of beta-catenin expression in breast cancer and its relationship with clinicopathologic parameters. *Asian Pac J Cancer Prev* **22**: 3493–3498. https://doi.org/10.31557/APJCP.2021.22.11.3493
Shawver LK, Slamon D, Ullrich A (2002) Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell* **1**: 117–123. https://doi.org/10.1016/s1535-6108(02)00039-9
Shen G, Gao Q, Liu F, Zhang Y, Dai M, Zhao T, Cheng M, Xu T, Jin P, Yin W, Huang D, Weng H, Chen W, Ren H, Mu X, Wu X, Hu S (2020) The Wnt3a/beta-catenin/TCF7L2 signaling axis reduces the sensitivity of HER2-positive epithelial ovarian cancer to trastuzumab. *Biochem Biophys Res Commun* **526**: 685–691. https://doi.org/10.1016/j.bbrc.2020.03.154
Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, Kaufmann M, Cameron D, Bell R, Bergh J, Coleman R, Wardley A, Harbeck N, Lopez RI, Mallmann P, Gelmon K, Wilcken N, Wist E, Sánchez Rovira P, Piccart-Gebhart MJ (2007) 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* **369**: 29–36. https://doi.org/10.1016/S0140-6736(07)60028-2
Song L, Liu D, He J, Wang X, Dai Z, Zhao Y, Kang H, Wang B (2016) SOX1 inhibits breast cancer cell growth and invasion through suppressing the Wnt/beta-catenin signaling pathway. *APMIS* **124**: 547–555. https://doi.org/10.1111/apm.12543

Tolaney SM, Tayob N, Dang C, Yardley DA, Isakoff SJ, Valero V, Faggen M, Mulvey T, Bose R, Hu J, Weckstein D, Wolff AC, Reeder-Hayes K, Rugo HS, Ramaswamy B, Zuckerman D, Hart L, Gadi VK, Constantine M, Cheng K, Briccetti F, Schneider B, Garrett AM, Marcom K, Albain K, DeFusco P, Tung N, Ardman B, Nanda R, Jankowitz RC, Rimawi M, Abramson V, Pohlmann PR, Van Poznak C, Forero-Torres A, Liu M, Ruddy K, Zheng Y, Rosenberg SM, Gelber RD, Trippa L, Barry W, DeMeo M, Burstein H, Partridge A, Winer EP, Krop I (2021) Adjuvant trastuzumab emtansine *versus* paclitaxel in combination with trastuzumab for stage I HER2-positive breast cancer (ATEMPT): a randomized clinical trial. *J Clin Oncol* **39**: 2375–2385. https://doi.org/10.1200/JCO.20.03398

Uijen MJM, Lassche G, van Engen-van Grunsven ACH, Driessen CML, van Herpen CML (2022) Case series of docetaxel, trastuzumab, and pertuzumab (DTP) as first line anti-HER2 therapy and ado-trastuzumab emtansine (T-DM1) as second line for recurrent or metastatic HER2-positive salivary duct carcinoma. *Oral Oncol* **125**: 105703. https://doi.org/10.1016/j.oraloncology.2021.105703

Wang HM, Xu YF, Ning SL, Yang DX, Li Y, Du YJ, Yang F, Zhang Y, Liang N, Yao W, Zhang LL, Gu LC, Gao CJ, Pang Q, Chen YX, Xiao KH, Ma R, Yu X, Sun JP (2014) The catalytic region and PEST domain of PTPN18 distinctly regulate the HER2 phosphorylation and ubiquitination barcodes. *Cell Res* **24**: 1067–1090. https://doi.org/10.1038/cr.2014.99

Wang Z, Li Z, Ji H (2021) Direct targeting of beta-catenin in the Wnt signaling pathway: Current progress and perspectives. *Med Res Rev* **41**: 2109–2129. https://doi.org/10.1002/med.21787

White BD, Chien AJ, Dawson DW (2012) Dysregulation of Wnt/beta-catenin signaling in gastrointestinal cancers. *Gastroenterology* **142**: 219–232. https://doi.org/10.1053/j.gastro.2011.12.001

Wu Y, Ginther C, Kim J, Mosher N, Chung S, Slamon D, Vadgama JV (2012) Expression of Wnt3 activates Wnt/beta-catenin pathway and promotes EMT-like phenotype in trastuzumab-resistant HER2-overexpressing breast cancer cells. *Mol Cancer Res* **10**: 1597–1606. https://doi.org/10.1158/1541-7786.MCR-12-0155-T

Wu Y, Tran T, Dwabe S, Sarkissyan M, Kim J, Nava M, Clayton S, Pietras R, Farias-Eisner R, Vadgama J v (2017) A83-01 inhibits TGF-beta-induced upregulation of Wnt3 and epithelial to mesenchymal transition in HER2-overexpressing breast cancer cells. *Breast Cancer Res Treat* **163**: 449–460. https://doi.org/10.1007/s10549-017-4211-y

Xie Y, Hung MC (1996) p66Shc isoform down-regulated and not required for HER-2/neu signaling pathway in human breast cancer cell lines with HER-2/neu overexpression. *Biochem Biophys Res Commun* **221**: 140–145. https://doi.org/10.1006/bbrc.1996.0559

Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT (1996) The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in Xenopus embryos by glycogen synthase kinase 3. *Genes Dev* **10**: 1443–1454. https://doi.org/10.1101/gad.10.12.1443

Zhang J, Gill AJ, Issacs JD, Atmore B, Johns A, Delbridge LW, Lai R, McMullen TP (2012) The Wnt/beta-catenin pathway drives increased cyclin D1 levels in lymph node metastasis in papillary thyroid cancer. *Hum Pathol* **43**: 1044–1050. https://doi.org/10.1016/j.humpath.2011.08.013

Zhang J, Zhang S, Li X, Zhang F, Zhao L (2021) HOXB5 promotes the progression of breast cancer through wnt/beta-catenin pathway. *Pathol Res Pract* **224**: 153117. https://doi.org/10.1016/j.prp.2020.153117

Zhang S, Huang WC, Li P, Guo H, Poh SB, Brady SW, Xiong Y, Tseng LM, Li SH, Ding Z, Sahin AA, Esteva FJ, Hortobagyi GN, Yu D (2011) Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med* **17**: 461–469. https://doi.org/10.1038/nm.2309

Zhang Y, Wang X (2020) Targeting the Wnt/beta-catenin signaling pathway in cancer. *J Hematol Oncol* **13**: 165. https://doi.org/10.1186/s13045-020-00990-3