WNT5B governs the phenotype of basal-like breast cancer by activating WNT signaling

Shaojie Jiang1†, Miaofeng Zhang2†, Yanhua Zhang3, Weiping Zhou4, Tao Zhu3, Qing Ruan3, Hui Chen5, Jie Fang6, Fei Zhou1*, Jihong Sun1* and Xiaoming Yang1,7*

Abstract

Background: Breast cancer is the leading cause of cancer-related death in women worldwide. Metastatic disease remains the primary cause of death in patients with breast cancer. Basal-like breast cancer (BLBC) is associated with aggressive behavior, stem-like phenotype, high histological grade, poor clinical features, and high rates of recurrences and/or metastasis. However, the mechanism of BLBC phenotype shaping remains obscure.

Methods: Seventeen normal breast/breast cancer cell lines were used for evaluating the breast cancer subtype-markers, WNT targets and constitutive components, and epithelial mesenchymal transition (EMT) markers analysis by western blot. One hundred and twenty formalin-fixed breast cancer tissues were used for immunohistochemistry (IHC) staining. Nine online platforms (cBioPortal, CCLE, GEPIA, etc.) were used for related analyses.

Results: We identified Wnt5b as a key regulatory factor that governs the phenotype of BLBC by activating canonical and non-canonical WNT signaling. Wnt5b exhibited basal-like specificity in cells and clinical samples both at the mRNA and protein levels and also showed good correlation with basal-like phenotype at the mRNA level. Besides, Wnt5b was also a promising therapeutic target for LGK-974 treatment. In addition, we identified that CK1α was expressed at low levels in BLBC and that the activation of CK1α by pyrvinium was an alternative strategy for BLBC treatment.

Conclusions: Wnt5b is not only a diagnostic biomarker but also a potential therapeutic target of BLBC.

Keywords: WNT5B, Basal-like breast cancer, Luminal breast cancer, Canonical/non-canonical Wnt signaling, Epithelial-mesenchymal transition

Background

Breast cancer is the most commonly diagnosed cancer (24.2% of the total cases) and the leading cause of cancer death (15% of the total cancer deaths) among females worldwide in 2018 [1]. Classification of breast cancer into luminal A, luminal B, Her-2+, and basal-like subtypes based on gene expression profiles has significantly changed the understanding and treatment of breast cancer [2–4]. Later, Basal-like breast cancer (BLBC) was defined as estrogen receptor (ER) negative, human epidermal growth factor receptor 2 (HER2) negative, epidermal growth factor receptor (EGFR, also known as Her-1) or/and Cytokeratin 5/6 (CK 5/6) positive [5].

A previous study reported 51% luminal A, 16% luminal B, 7% Her-2+, 20% basal-like, and 6% normal-like breast cancers in 496 cases of invasive breast cancer [6]. Thus, BLBC is the second most common subtype after luminal A of invasive breast cancer. BLBC is often associated with metastatic disease and the incidence of metastasis in the BLBC is second to the Her-2-enriched subtype. Notably, BLBC has the shortest median survival (0.5 years) among all subtypes with distant metastasis [7]. Thus, BLBC is the most fatal subtype among breast cancer subtypes. Previous studies demonstrated that BLBC cell lines expressed EMT-acquired markers such as Snail, vimentin, and N-cadherin, but lost EMT-attenuated marker E-cadherin compared to luminal breast

* Correspondence: xmyang@zju.edu.cn; xmyang@uw.edu; sunjihong@zju.edu.cn; srrheizhou@zju.edu.cn
† Shaojie Jiang and Miaofeng Zhang contributed equally to this work.
1Department of Radiology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, Zhejiang, China
2Full list of author information is available at the end of the article

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
cancer cell lines [8, 9]. Various studies have indicated that both the canonical and non-canonical Wnt signaling is involved in the metastasis of breast cancer via the EMT pathway [9–12]. However, whether canonical or non-canonical Wnt signaling contributes to the metastasis of all breast cancers subtypes is relatively unclear.

Canonical Wnt signaling is associated with the metastasis of various cancers, including breast cancer [13]. Wnt ligands and Wnt constructive components are frequently mutated, and over- or under-expressed in many different human cancers – in particular, adenomatous polyposis coli (APC) in colorectal cancer [14], CTNNB1 (encoding β-Catenin) in colon adenocarcinoma [15], lung adenocarcinoma [16], and endometrial carcinoma [17], and WTX (Wilms tumor gene on the X chromosome, also known as FAM123B) in Wilms tumor [18], etc. are the most commonly dysregulated canonical Wnt elements. However, no or very few CTNNB1 mutations have been reported in breast cancer [19]. In addition, the subcellular localization of β-Catenin differs in breast cancer subtypes – invasive ductal carcinomas exhibited membranous β-Catenin staining, BLBC exhibited strong nuclear β-Catenin staining, while lobular carcinomas lacked β-Catenin expression [13, 19].

Non-canonical Wnt signaling is grouped into several categories including Wnt/planar cell polarity (PCP), Wnt-cGMP/Ca2+, Wnt-RAP1, and Wnt-ROR2 (Receptor tyrosine kinase-like orphan receptor 2). All these types of non-canonical Wnt signaling are characterized as Wnt- or Frizzled (Fzd)-initiated but β-Catenin independent [20]. Wnt5a is a non-canonical Wnt ligand which is overexpressed specifically in BLBC cell lines, and the inhibition of the Twist-bromodomain containing 4 (BRD4) association by JQ1 reduced Wnt5a expression and suppressed invasion, cancer stem cell (CSC)-like property, and tumorigenicity of BLBC cells [21]. Fibroblast-secreted exosomes promote the protrusive activity and motility of breast cancer cells through Wnt-PCP signaling [10]. Non-canonical Wnt receptor Fzd2 and its ligands Wnt5a/b are elevated in metastatic breast cancer cell lines and in high-grade tumors and that their expression correlates with markers of EMT [11].

However, the mechanisms by which the BLBC cells maintain their physical and physiological phenotype remain obscure. In this study, we examined the factors affecting the physical and physiological phenotype of BLBC cells and identified WNT5B as a key factor required for shaping the phenotype of BLBC cells.

Methods

Cell lines

All breast cell lines were purchased from the CBCAS (Cell Bank of the Chinese Academy of Sciences, Shanghai, China). All cell lines except Bcap-37 were maintained in culture as described in a previous report [22]. Bcap-37, a Chinese breast cancer cell line established by Changwei Chen (J. Beijing Medical College, 1983, 15(3): 161), was maintained in RPMI 1640 medium (Gibco, 11,875–085) with 10% fetal bovine serum (Gibco, 1099–141). The cell lines were divided into the normal group, luminal group, and basal-like group based on a previous study [22].

Western blot analysis

All cell lines were harvested at ~ 80% confluence and then lysed using RIPA buffer (Thermo Scientific, #89901). Protein concentration was measured using the BCA protein assay kit (Thermo Scientific, #23225). Protein samples were resolved by 8–12% SDS-PAGE and transferred to a PVDF membrane (Bio-Rad, #162–0117). The membrane was blocked in TBS containing 0.05% Tween-20 (Amresco, 0777-1 L) with 5% non-fat skim milk (BD, #232100) for 1 h at 25 °C, followed by overnight incubation with primary antibodies overnight at 4 °C. After three washes in TBST, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at room temperature. After three washes in TBST, the membrane was washed to wash the EZ-ECL kit (Biological Industries, #20–500-120) reagent, and visualized using a Tanon-5200 multi-automatic chemiluminescence/fluorescence imaging analysis system (Tanon Science & Technology Inc., Shanghai, China).

Antibodies for western blot

Canonical breast cancer subtypes markers: anti-Her-2/ErbB2 (CST, #2165), anti-Progesterone Receptor A/B (CST, #8757), anti-Estrogen Receptor α (CST, #8644), anti-Keratin 18 (CST, #4548), anti-FoxA1/HNF3α (CST, #58613), anti-AGR2 (CST, #13062), anti-CD44 (CST, #3570), anti-Caveolin-1 (CST, #3267), anti-Caveolin-2 (CST, #8522), anti-EGF Receptor (CST, #4267), anti-Cytokeratin 5 (Sigma, SAB5300267), and anti-SPARC (CST, #5420).

Wnt signaling targets: anti-Met (CST, #8198), anti-CD44 (CST, #3570), anti-TCF1/7 (CST, #203), anti-c-Jun (CST, #9165), anti-LEF1 (CST, #2230), anti-c-Myc (CST, #5605), anti-Cyclin D1 (CST, #2978), anti-MMP-7 (Abcam, ab207299), and anti-Axin2 (CST, #2151).

Canonical Wnt signaling constitutive components: anti-Phospho-LRP6 (Ser1490) (CST, #2568), anti-LRP6 (CST, #3395), anti-Dvl2 (CST, #3224), anti-Dvl3 (CST, #3218), anti-APC (CST, #2504), anti-Axin1 (CST, #2087), anti-CK1α (Santa cruz, sc-6477), anti-GSK-3α/β (CST, #5676), anti-Non-phospho (Active) β-Catenin (Ser45) (CST, #19807), anti-Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (CST, #8814), anti-Phospho-β-
Catenin (Ser552) (CST, #5651), anti-Phospho-β-Catenin (Ser675) (CST, #4176), anti-Phospho-β-Catenin (Thr41/Ser45) (CST, #9565), anti-Phospho-β-Catenin (Ser33/37/Thr41) (CST, #9561), and anti-β-Catenin (CST, #8480).

EMT markers: anti-E-cadherin (CST, #3195), anti-ZO-1 (CST, #8193), anti-N-cadherin (CST, #13116), anti-Claudin-1 (CST, #13255), anti-β-Catenin (CST, #8480), anti-Vimentin (CST, #5741), anti-TCF8/ZEB1 (CST, #3396), anti-Snail (CST, #3879), anti-Slug (CST, #9585), and anti-TWIST1 (CST, #46702).

Non-canonical Wnt signaling components: anti-Phospho-JNK (CST, #9251), anti-JNK (CST, #9252), anti-Phospho-Stat3 (Tyr705) (CST, #9145), anti-Stat3 (CST, #12640), and anti-Erk1/2 (CST, #9102).

Other antibodies: anti-Wnt3a (Abcam, ab81614), anti-Wnt5a (HUABIO, #ET1706), anti-Wnt5b (Abcam, ab124818), anti-β-Actin (CST, #3700), secondary antibodies including anti-rabbit IgG, HRP-linked Antibody (CST, #7074), and anti-mouse IgG, HRP-linked Antibody (CST, #7076). All primary antibodies that were used for western blot were diluted into 1: 1000, and secondary antibodies that were used for western blot were diluted into 1: 2000.

Clinical specimens, IHC, and immunofluorescence (IF) staining

Formalin-fixed and paraffin-embedded primary breast cancer tissues were obtained from Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University. Molecular subtypes were histologically characterized by three pathologists. All human tissues were obtained with written informed consents and with the approval of the Medical Ethical Committee of Sir Run Run Shaw Hospital. IHC staining was performed following our previously reported protocol [23]. The following antibodies were used for IHC staining – anti-Her-2/ErbB2 (Roche, 790–4493), anti-PR (Novocastra, NCL-L-PGR-312), anti-ER (Novocastra, NCL-L-ER-6F111), anti-Ki-67 (Dako, M7240), anti-EGFR (Roche, 790–4347), anti-CK-5/6 (Dako, M7237), anti-Wnt5b (Abcam, ab94914), and REAL™ EnVision™ Detection System, HRP/DAB, Rabbit/Mouse kit (Dako, K5007). The other antibodies were the same as those used for western blot. Anti-β-Catenin (CST, #8480), Anti-rabbit IgG (H+L), F (ab’)2 Fragment (Alexa Fluor® 555 Conjugate) (CST, #4413), DyLight™ 554 Phalloidin (CST, #13054), and DAPI Staining Solution (Beyotime, C1006) were used for IF staining, which was performed according to the protocol from (https://www.cellsignal.com/). Breast cancer cells were treated with or without recombinant human Wnt3a (200 ng/mL, R&D, 5036-WN/CF), Wnt5a (500 ng/mL, R&D, 645-WN/CF), or Wnt5b (500 ng/mL, R&D, 7347-WN/CF) protein for 24 h before IF staining. All primary antibodies that were used for IHC/IF were diluted into 1: 200, and secondary antibodies that used for IHC/IF were diluted into 1: 2000.

Bioinformatic analysis based on online platforms

mRNA expression of the indicated genes among basal-like, Her-2+, luminal A, luminal B, and normal-like breast cancer patients according to Sørlie’s subtypes [3, 4] was assessed by Breast Cancer Gene-Expression Miner v4.1 (bc-GenExMiner v4.1) (http://bcgenex.centregauducheau.fr) [24, 25]. The P value was also calculated by bc-GenExMiner v4.1. mRNA expression of the indicated genes among normal, Her-2+, luminal and triple negative breast cancer (TNBC) patients were assessed by UALCAN (http://ualcan.path.uab.edu/) [26]. The P value was also calculated by UALCAN.

A total of 6370 sequenced cases/patients from 11 invasive breast carcinoma studies were selected for amplification (AMP) or copy-number alteration (CNA) analyses from the cBio Cancer Genomics Portal (cBioPortal) (http://www.cbiopiortal.org/) [27, 28]. The survival analysis between the Myc or/and CCND1 AMP group and the Myc/CCND1 normal group was also obtained from the cBioPortal.

WNT3A, WNT5A, and WNT5B mRNA expression levels across various normal tissues were derived from the Genotype-Tissue Expression (GTex) (https://gtexportal.org/) [29] which deposited in the Human Protein Atlas (HPA) (https://www.proteinatlas.org/) [30].

Breast cancer samples from patients were divided into WNT3, WNT5A, WNT5B, WNT11-high and WNT3, WNT5A, WNT5B, WNT11-low-expression groups by auto-cutoff according to the mRNA expression value, and the prognosis of overall survival (OS) and relapse free survival (RFS) were assessed by Kaplan-Meier Plotter (KM-plotter) (http://kmplot.com/) [31]. The P value was also calculated by KM-plotter.

mRNA expression data of the indicated genes in heat-maps were extracted from the Cancer Cell Line Encyclopedia (CCLE) (https://portals.broadinstitute.org/ccle) [32] and displayed using GraphPad Prism 7 software. Thirty-four breast cancer lines were divided into a luminal group and a basal-like group according to Sørlie's subtypes [3, 4] was assessed by Breast Cancer Gene-Expression Miner v4.1 (bc-GenExMiner v4.1) (http://bcgenex.centregauducheau.fr) [24, 25]. The P value was also calculated by bc-GenExMiner v4.1. mRNA expression of the indicated genes among normal, Her-2+, luminal and triple negative breast cancer (TNBC) patients were assessed by UALCAN (http://ualcan.path.uab.edu/) [26]. The P value was also calculated by UALCAN.

Correlation between WNT5B or CSNK1A1 and other indicated genes according to the mRNA expression value was calculated by GEPIA (http://gepia.cancer-pku.cn/) [33]. The P value and R-value were also calculated by GEPIA.

Protein-Protein interaction (PPI) pattern of WNT3A, WNT5A, and WNT5B was based on STRING (https://string-db.org/) [34].
Fig. 1 (See legend on next page.)
Briefly, a 10-cm dish of $4 \times 10^6$ non-confluent 293 T cells expressing levels of selected canonical breast cancer subtype-marker mRNAs with Her-2 positive, luminal or basal-like specific based on bcGenExMiner v4.1 according to the Sørlie's subtypes (*$p < 0.05$; ****$p < 0.0001$; Red star: the former > the latter; Green Star: the former < the latter). Expression of selected luminal breast cancer markers (c) and basal-like specific marker Caveolin-1 (d) in representative Her-2 positive, luminal A, luminal B and basal-like breast cancer tissues by IHC (Red scale bar = 200 μm; Purple scale bar = 40 μm). e The association of selected luminal or basal-like specific markers expression between basal-like and non-BlBC tissue subtypes were assessed using Pearson’s $\chi^2$ test (for CK18, $p = 0.0076$; for FoxA1, $p < 0.0001$; for AGR2, $p = 0.0002$; for Caveolin-1, $p < 0.0001$; Red star, basal-like > non-basal-like; Green star, basal-like < non-basal-like).

**Fig. 1** Expression of canonical breast cancer subtype-markers in breast cancer cells and clinical samples. a Expression of canonical breast cancer subtype-markers in two normal breast cell lines, eight luminal, and seven BlBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected luminal breast cancer markers and basal-like specific marker Caveolin-1 in representative Her-2 positive, luminal A, luminal B and basal-like breast cancer tissues by IHC (Red scale bar = 200 μm; Purple scale bar = 40 μm). c Semi-RT-PCR products were analyzed by electrophoresis on a 1% agarose gel. Primer sequences (F, forward; R, reverse; 5′➔3′) are listed below.

**Virus production and transfection**

shRNAs targeting WNT5B were cloned into the pLent-U6-RFP-Puro (Vigne, #LT88024) vector. Targeting sequences were as follows:

- shRNA-Ctr: 5′-GCACCCAGUCCGCC CUAGACAA-3′,
- shWNT5B-1: 5′-GGAAGGAAGGCAGCU UUUUA-3′,
- shWNT5B-2: 5′-GUGGACCAGUACAU CGUGUAAA-3′,
- shWNT5B-3: 5′-GAAUUGCAGACAGC GGACAA-3′.

The 293 T cell line was used for lentivirus packaging. Briefly, a 10-cm dish of $4 \times 10^6$ non-confluent 293 T cells was co-transfected with recombinant pLent-U6-RFP-Puro, pMD2G (Addgene, #12259), and psPAX2 (Addgene, #12260). The lentivirus-containing supernatant was harvested after 48 h and used for the subsequent experiment.
nu mice (5 weeks old, female; 4–5 mice per group). The tumor size was measured every 3 days for Bcap-37 and 5 days for MDA-MB-231, and tumor volume was calculated using the formula \((\text{length} \times \text{width} \times \text{width})/2\). When tumors reached a 1000 mm\(^3\) size the mice were sacrificed. Images of breast cancer cell-bearing mice were acquired using a Clairvivo OPT plus imaging system (SHIMADZU). Briefly, mice were anesthetized using 2% isoflurane for 5 min using the RC2 Anesthesia Machine (VetEquip). The mice were then imaged under 530 nm laser irradiation. Ultrasound imaging was performed following our previously reported protocol [23].

**Statistical analyses**

All data were analyzed using GraphPad Prism 7 software and are represented as the mean ± standard error of the
Fig. 3 (See legend on next page.)
mean (SEM) unless otherwise indicated. Statistical analyses were performed using an unpaired Student’s t-test. For the tumor growth curve, data were assessed by two-way ANOVA method, and $P < 0.05$ was considered to indicate statistical significance. For the IHC data, data were assessed by Pearson’s $\chi^2$ test. For all comparisons, $P < 0.05$ was considered to indicate statistical significance.

**Results**

**Analyses of biomarkers of breast cancer subtypes in normal breast/breast cancer cell lines and clinical samples**

We collected two normal breast cell lines, eight luminal, and seven BLBC cell lines. Her-2 as a marker for Her-2+ breast cancer; PR, ER, CK18 (Cytokeratin 18), FoxA1 (also called HNF3α), and AGR2 (Anterior gradient 2) as markers of luminal breast cancer; CD44, Caveolin-1, Caveolin-2, EGFR, CK5 (Cytokeratin 18), a BLBC cell line for the first time. However, Caveolin-1, Caveolin-2, and SPARC did not exhibit specificity based on bc-GenExMiner. As expected, E-cadherin, an attenuated EMT marker was lost in most of the BLBC cell lines, while most acquired EMT markers were preferentially expressed in BLBC cell lines, especially N-cadherin, vimentin, ZEB1, Snail, Slug, and Twist1 were selected as EMT markers based on a previous review [37].

**Analyses of EMT biomarkers in normal breast/breast cancer cell lines and clinical samples**

Loss of attenuated EMT markers but overexpression of acquired EMT markers is another property of BLBC [8, 9]. E-cadherin, ZO-1, N-cadherin, Claudin-1, β-Catenin, vimentin, ZEB1, Snail, Slug, and Twist1 were selected as EMT markers based on a previous review [37]. As expected, E-cadherin, an attenuated EMT marker was lost in most of the BLBC cell lines, while most acquired EMT markers were preferentially expressed in BLBC cell lines, especially N-cadherin, vimentin, ZEB1, Slug, and Twist1 (Fig. 2a). CDH1 (encoding E-cadherin) was expressed at a low level, while CLDN1 (encoding Claudin-1) and VIM (encoding vimentin) were highly expressed in BLBC (Fig. 2b) and TNBC (Fig. 2c). We next examined the expression of E-cadherin and vimentin in breast cancer clinical samples and observed similar results (Fig. 2d and e).

**Most canonical Wnt signaling targets are preferentially overexpressed in BLBC**

A total of 6370 sequenced cases/patients from 11 invasive breast carcinoma studies were selected for AMP analysis based on cBioPortal. Wnt ligands, such as WNT3A, WNT9A, WNT11 and WNT5B were highly amplified in breast cancers (Fig. 3a). Further, Myc (encoding c-Myc) and CCND1 (encoding Cyclin D1), AXIN2, etc. as canonical Wnt signaling targets were highly amplified in breast cancers (Fig. 3b). Moreover, breast cancer with Myc and/or CCND1 exhibited poorer overall prognosis (Fig. 3c). So, we examined the expression of c-Myc and Cyclin D1, as well as other Wnt targets in the indicated cell lines. We found that most canonical Wnt signaling targets, especially Met, CD44, and TCF1/7 were preferentially overexpressed in BLBC cell lines. Surprisingly, Cyclin D1 was preferentially overexpressed in luminal breast cancer cell lines (Fig. 3d). Similarly, Met, CD44, TCF1/7, Myc, and MMP-7 were preferentially overexpressed in BLBC and TNBC, while Cyclin D1 showed luminal-specificity at the mRNA level (Fig. 3e and Additional file 1: Figure S2a). Furthermore,
Met, CD44, and TCF1/7 were also preferentially expressed in BLBC clinical samples while Cyclin D1 was preferentially expressed in luminal breast cancer clinical samples (Fig. 3f and g).

**Fig. 4** WNT5B is a specific marker of BLBC with poor prognosis. 

**a** mRNA expression of WNT ligands and their receptors Frizzleds between luminal and BLBC cell lines are shown in the heatmap (data are extracted from CCLE and displayed by using GraphPad Prism 7 software). 

**b** mRNA and protein expression levels of WNT3A, WNT5A, and WNT5B in two normal breast cell lines, eight luminal, and seven BLBC cell lines by semi-q RT-PCR and western blot (S: Short exposure; L: Long exposure).

**c** Expression of Wnt5a and Wnt5b in representative Her-2 positive, luminal A, luminal B and BLBC tissues by IHC (Red scale bar = 200 μm; Purple scale bar = 40 μm).

**d** The association of Wnt5a or Wnt5b expression between basal-like and non-BLBC tissue subtypes were assessed using Pearson’s χ² test (**p = 0.0003, ****p < 0.0001). Prognostic value of WNT5A (e) and WNT5B (f) mRNA levels in human breast cancer, data obtained from the KM-plotter.

WNT5B is a specific marker of BLBC with poor prognosis

To explain why the Wnt targets (especially Met, CD44, and TCF1/7) are preferentially expressed in BLBC. We first selected 34 breast cancer cell lines with known
Fig. 5 (See legend on next page.)
molecular typing [22], and analyzed the expression of Wnt ligands and their receptors based on CCLE. We found that WNT3, WNT5A, WNT5B and WNT11 were preferentially overexpressed in BLBC cell lines at mRNA level (Fig. 4a). In view of (Fig. 3a), we analyzed the expression of WNT3A, WNT3, WNT5A, WNT5B and WNT11 in bc-GenExMiner and UALCAN, and found that WNT5B was preferentially overexpressed in both BLBC and TNBC at mRNA level, while WNT11 was preferentially overexpressed in BLBC but not TNBC (Additional file 1: Figure S2f). We further examined the expression of WNT3A, WNT5B and WNT11 in bc-GenExMiner and UALCAN, and found that WNT5B was preferentially overexpressed in both BLBC and TNBC at mRNA level, while WNT11 was preferentially overexpressed in BLBC but not TNBC (Additional file 1: Figure S2h & c). Next, we analyzed the OS and RFS between WNT3/11-low and WNT3/11-high group by using KM-plotter. WNT3-high group showed good prognosis of OS and RFS (Additional file 1: Figure S2d), while WNT11 exhibited good prognosis of OS but not RFS (Additional file 1: Figure S2e). Therefore, WNT3 and WNT11 were not the key factors for BLBC phenotype shaping, while WNT5B exhibited BLBC and TNBC specificity. WNT3A is the most well-known canonical Wnt signaling ligand, and was used as a control ligand in the following study. WNT5A has been proved as an important factor for maintaining the cancer stem cell (CSC)-like, tumorigenicity and BLBC phenotype in BLBC cells, was also used in the following study [21].

We Next analyzed the expression of WNT3A, WNT5A, and WNT5B across various normal tissues based on GTEx, and found that WNT3A was not expressed in normal breast tissues, while WNT5A and WNT5B were expressed in normal breast tissues (Additional file 1: Figure S2f). We further examined the expression of WNT3A, WNT5A, and WNT5B in the indicated cell lines at both the mRNA and protein levels, and found that WNT3A was also not expressed in in breast cancer cell lines, while WNT5A and WNT5B were preferentially expressed in BLBC cell lines (Fig. 4b) (note: normal breast cell lines HBL-100 and MCF-10A also belong to the basal-like type [22]). Moreover, Wnt5a and Wnt5b were preferentially expressed in BLBC clinical samples (Fig. 4c and d). Higher expression of WNT5B, but not WNT5A correlated with poorer prognosis (Fig. 4e and f). Thus, WNT5B is a potential biomarker of BLBC.

Besides, we examined the expression of Wnt constitutive components, including LDL receptor-related protein 6 (LRP6), disheveled segment polarity protein 2 (DVL2), DVL3, APC, Axin1, Casein kinase 1α (CK1α), GSK-3β, active β-Catenin (Np-Ser45, Np-Ser33/37/Thr41, p-Ser552, p-Ser675), inactive β-Catenin (p-Thr41/Ser45, p-Ser33/37/Thr41), and total β-Catenin in the indicated cell lines. We found that CK1α was specifically low expressed in BLBC cell lines. However, β-Catenin expression was not significantly different between the luminal group and basal-like group (Additional file 1: Figure S3a). Meanwhile, LRP6 and DVL3 were preferentially overexpressed, while CSNK1A1 (encoding CK1α) was preferentially low-expressed in BLBC or TNBC at the mRNA level (Additional file 1: Figure S3b & c). Total β-Catenin and active β-Catenin (Np-Ser45, Np-Ser33/37/Thr41) expressions were also not obviously different between Her-2+, luminal A, luminal B, and BLBC tissues (Additional file 1: Figure S3d). Similarly, β-Catenin was located in cell membrane in both luminal and basal-like cancer cell lines (Additional file 1: Figure S3e). These results suggested that canonical Wnt/β-Catenin is not the crucial signaling pathway for BLBC phenotype shaping.

**WNT5B is positively correlated with basal-like phenotype, and inversely correlated with luminal phenotype**

To explore the correlation between WNT5B and basal-like phenotype, we selected 34 breast cancer cell lines with known molecular typing [22] based on CCLE. As expected, WNT5B, Wnt targets (MET, CD44, TCF7, and MMP7), acquired EMT markers, and basal-like markers were preferentially expressed in BLBC cell lines (Fig. 5a). Then we analyzed the correlation between WNT5B and Wnt targets, EMT, luminal, and basal-like markers, and found that WNT5B was positively correlated with most Wnt targets, except LEF1 and CCND1 (CCND1 showed luminal-specificity) (Fig. 5b). Besides, WNT5B was positively correlated with most acquired EMT markers but was inversely correlated with attenuated EMT marker (CDH1) (Fig. 5c). Notably, WNT5B was inversely correlated with all luminal markers (Fig. 5d) but positively correlated with all basal-like markers (Fig. 5e). Thus, WNT5B is a potential factor for shaping the phenotype of BLBC cells.

**WNT5B governs the phenotype of BLBC by activating canonical and non-canonical Wnt signaling**

Typical BLBC cell lines: Hs-578T, MDA-MB-231 and newly identified Bcap-37 were chosen for subsequent
Fig. 6 (See legend on next page.)
Lentivirus-mediated shRNA was used to knockdown Wnt5b expression in Hs-578 T, MDA-MB-231, and Bcap-37 cells. sh-WNT5B-2 and WNT5B-3 showed high efficiency of knockdown and were chosen for further studies (Fig. 6a). Recombinant Wnt5b promoted the migration and invasion of Bcap-37 and MDA-MB-231 cells, while the knockdown of endogenous Wnt5b inhibited migration and invasion of Bcap-37 and MDA-MB-231 cells. These suggested that Wnt5b is an important factor for BLBC cell migration and invasion (Fig. 6b and c). Recombinant Wnt5b upregulated active and total β-Catenin slightly similar to Wnt3a and Wnt5a (Fig. 6d); in addition, it also activated non-canonical Wnt signaling by promoting phosphorylation of JNK, Erk1/2, and Stat3 significantly as with Wnt5a (Fig. 6e). Active and total β-Catenin was reduced in Wnt5b-KD cells, and the phosphorylation of JNK, Erk1/2, and Stat3 were reduced in Wnt5b-KD cells (Fig. 6f). Besides, luminal markers FoxA1 and CK18 were upregulated while basal-like markers EGFR, CD44, SPARC, Caveolin-1, and Caveolin-2 were reduced in Wnt5b-KD cells (Fig. 6g). Acquired EMT markers ZEB1, vimentin, Snail, and Slug were also reduced in Wnt5b-KD cells (Fig. 6h). Recombinant Wnt5b altered the cells to exhibit a more mesenchymal-type morphology similar to Wnt5a (Fig. 6i). Stat3 was persistently phosphorylated in BLBC cells [38], so we next examined the expression of p-Stat3 in clinical samples, and found that p-Stat3 was preferentially expressed in BLBC as expected (Fig. 6j and k). These evidences suggested that WNT5B governs the phenotype of BLBC by activating both canonical and non-canonical Wnt signaling, and non-canonical Wnt is the dominant signaling pathway for BLBC phenotype shaping.

Knockdown of WNT5B inhibits the tumorigenicity of basal-like cancer cells in vivo
Lentivirus-mediated shRNA also expressed red fluorescent protein (RFP) (Fig. 6a), and the cells that were infected with the lentivirus showed red fluorescence under visible light (Fig. 7a and b). We used RFP to monitor tumor growth, and found that knockdown of Wnt5b inhibited the tumor growth compared to the control (Fig. 7c). Ultrasound imaging further confirmed that the tumor growth after knockdown of WNT5B was significantly inhibited (Fig. 7d). In addition, invalid RNA interference (sh-WNT5B-1) in MDA-MB-231 cells did not inhibit tumor growth, and it was also observed in Bcap-37 cells. The inhibition efficiency of tumor growth is proportional to the efficiency of RNA interference (Fig. 7e-l). These evidences suggested that Wnt5b is not only a diagnostic biomarker but also a potential therapeutic target of BLBC.

Wnt5b is a potential therapeutic target of BLBC
Wnt undergoes post-translational acylation (palmitoylation) mediated by Porcupine, a membrane-bound O-acyltransferase [39] (Additional file 1: Figure S4a). LGK-974 (also known as WNT-974, a Phase I drug for TNBC and other Wnt ligands-dependent cancers) is a Porcupine inhibitor and can inhibit Wnt5a and Wnt5b secretion effectively [40]. On the other hand, CK1α, a canonical Wnt signaling inhibitor showed low-expression in BLBC (Additional file 1: Figure S3a), and was positively correlated with luminal markers and some attenuated EMT markers and was inversely correlated with some basal-like, acquired EMT markers and WNT5B (Additional file 1: Figure S4b-g), pyrvinium is a potent inhibitor of Wnt signaling which acts by activating CK1α [41]. Therefore, LGK-974 and pyrvinium were selected for further studies (Fig. 8a). Hs-578 T with HRAS mutant (G12D) and Wnt5a high/Wnt5b high was more sensitive to LGK-974 than Bcap-37 (Fig. 8b) and MDA-MB-231 with KRAS mutant (G13D) was more sensitive to pyrvinium than Bcap-37 (Fig. 8c). In addition, the foci formation assay showed that Hs-578 T was more sensitive to LGK-974 and pyrvinium than Bcap-37, and Wnt5b reversed the foci formation partly (Fig. 8d and e). Moreover, LGK-974 and pyrvinium also inhibited the tumor growth in Bcap-
Fig. 7 (See legend on next page.)
37 cells bearing mice xenograft model (Fig. 8f-j and Additional file 1: Figure S4h).

The reduction of the proliferation marker Ki-67 was also observed in the xenograft tumors (Fig. 8k and Additional file 1: Figure S4i). In summary, Wnt5b governs the phenotype of BLBC by activating both canonical and non-canonical Wnt signaling (Additional file 1: Figure S5), and should be a promising theranostic target of basal-like breast cancer.

**Discussion**

BLBC is associated with aggressive behavior, stem-like phenotype, high histological grade, poor clinical features, and high rates of recurrences and/or metastasis [42]. However, the mechanisms shaping the phenotype of BLBC remain obscure. In this study, we provide several mechanistic insights on how Wnt5b governs the phenotype of BLBC.

Wnt signaling activation has been observed in breast, lung, and hematopoietic malignancies and contributes to tumor recurrence [43], and is a promising therapeutic target for breast cancer therapy [44]. However, the relationship between Wnt signaling and breast cancer is still largely unknown, especially with regard to the molecular type of breast cancer. We analyzed the sequenced invasive breast carcinoma cases/patients from cBioPortal and found that Myc and CCND1 were highly amplified at the DNA level in breast cancer. We further found that most Wnt targets were preferentially expressed in BLBC, except Cyclin D1, which exhibited luminal-specificity. Then, we analyzed the canonical Wnt signaling constitutive components, but no significant differences were found between basal-like and luminal breast cancers, especially the various forms of β-Catenin, although CK1α expressed at low levels was identified in BLBC. Surprisingly, the unchanged β-Catenin induced activation of Wnt targets in BLBC. Thus, canonical Wnt signaling is not the leading factor for basal-like phenotype shaping, and there must be another mechanism.

Previous studies indicated that non-canonical Wnt ligands Wnt5a and Wnt5b were preferentially expressed in BLBC [11, 21, 45, 46], or TNBC [47]. Our results were in line with these previous reports and we found that Wnt5a and Wnt5b were preferentially expressed in BLBC; in addition, Wnt5b was superior to Wnt5a for molecular typing, as well as in prognosis estimation. BLBC has a high incidence of metastasis and shows high expression of acquired EMT markers [9, 21]. Wnt5a and Wnt5b have been proven as regulators of EMT in breast cancer [21, 45], and we also corroborated this conclusion. Moreover, we found that Wnt5b is positively correlated with basal-like phenotype, but inversely correlated with luminal phenotype for the first time, and also demonstrated that Wnt5b governs the phenotype of BLBC by activating canonical and non-canonical Wnt signaling. These results are consistent with the findings of previous studies [21, 48, 49].

A recent study demonstrated that Wnt5b promotes cancer cell migration and proliferation by exosome-mediated secretion. Knockdown of Wnt5b decreased the exosome-mediated secretion and inhibited Wnt5b-dependent cell proliferation and migration [49]. Therefore, Wnt5b may be a promising target for BLBC treatment. We further knocked down Wnt5b in MDA-MB-231 and Bcap-37 cells, which significantly inhibited the tumor growth.

The lentivirus transfected cells also expressed RFP and the quantification of the red fluorescence using external fluorescence imaging correlated strongly with tumor volume as reported previously [50, 51]. Thus, RFP is useful for superficial tumor growth monitoring for cancer cells with Wnt5b-KD or treated with small molecules.

Surprisingly, CK1α, a Wnt signaling inhibitor [52], was expressed at a higher level in luminal cancers than in BLBC cell lines. Therefore, Wnt5b and CK1α were selected as therapeutic targets. LGK-974, a Porcupine inhibitor inhibited Wnt5a and Wnt5b secretion effectively [40], and was chosen for Wnt5b targeted treatment, while pyrvinium, an activator [41] of CK1α was also chosen for CK1α targeted treatment, although the various phosphorylated states (Ser-45) of β-Catenin showed no significant difference between luminal and BLBC cell lines. Hs-578 T with HRAS mutant (G12D) and Wnt5a-high/ Wnt5b-high was more sensitive to LGK-974 than Bcap-37, and exogenous Wnt5b attenuated the effect of LGK-974 to a certain extent as expected. The results further strengthen the evidence for the clinical utility of LGK-974.

MDA-MB-231 with KRAS mutant (G13D) was more sensitive to pyrvinium than Bcap-37, but total or active...
Fig. 8 (See legend on next page.)
β-Catenin was expressed in MDA-MB-231 and Bcap-37 at a very low level. In fact, CK1α is a multifunctional protein involved in various signaling pathways [52] including Wnt/β-catenin, Hedgehog, autophagy, NF-κB, etc. Activation of CK1α by pyrvinium also inhibited the proliferation and tumor growth via attenuation of the Hedgehog signaling pathway [53] or inhibition of autophagosomal formation [54]. Previous studies reported that aggressive breast cancer cell lines SUM-149 and SUM-159 (both belong to the basal-like type [22]) were inhibited in vitro and in vivo [55]. However, RAS mutant cells seem more sensitive to LGK-974 and pyrvinium. Bcap-37 xenograft tumors treated with LGK-974 and pyrvinium showed more sensitivity to LGK-974 and pyrvinium. Bcap-37 xenograft tumors treated with LGK-974 and pyrvinium showed more sensitivity to LGK-974 and pyrvinium.

**Conclusions**

1. **Chemical structure of LGK-974 and pyrvinium pamoate.** Cell viability was determined in MDA-MB-231 and Bcap-37 cells following treatment with LGK-974 (b) and pyrvinium pamoate (c) for 72 h. Mean ± SEM is shown (assays performed in quadruplicate).
2. **LGK-974 and pyrvinium pamoate inhibited cell lines with a high expression of Wnt5b and a low expression of CK1α by foci formation assay.**
3. **Graphs represent LGK-974-treated or pyrvinium pamoate-treated Hs-578 T and Bcap-37 with or without Wnt5b (500 ng/ml), mean ± SD (n = 3 per treatment group), (n.s.: no significant, *p < 0.05, **p < 0.005, ***p < 0.001, ****p < 0.0001).**
4. **Images of Bcap-37 cells bearing tumors under 530 nm laser irradiation at day 5, 10, and 15 following treatment with LGK-974 (5 mg/kg) or pyrvinium pamoate (1 mg/kg).**
5. **Representative images of Ki-67 staining by IHC after 14 days of treatment.**

**Additional file**

**Figure S1.** Expression of canonical breast cancer subtype-markers in breast cancer. a Expression of selected canonical breast cancer subtype-marker mRNAs with Her-2 positive-specific, luminal-specific, or TNBC-specific based on UALCAN (Red p: the former > the latter; Green p: the former < the latter). b Common breast cancer subtype-marker expression in representative Her-2 positive (Her-2+), luminal A (ER/PR+, Her-2-, EGFR, Ki-67% < 14%), luminal B (ER/PR+, Her-2-, EGFR, Ki-67% ≥ 15%), and BLBC (ER-, Her-2-, EGFR+, CK5/6+) tissues by IHC. (Red scale bar = 200 μm; Purple scale bar = 40 μm). **Figure S2.** Identification of BLBC-specific Wnt ligands based on online platforms. a mRNA expression of selected canonical Wnt signaling targets with TNBC or non-luminal-specific based on UALCAN (Red p: the former > the latter; Green p: the former < the latter). b mRNA expression of WNT3A, WNT3, WNT5A, WNT5B, and WNT11 in five different breast cancer subtypes based on bc-GenExMiner v4.1 according to the Sørlie’s subtypes. (***p < 0.001; Red star: the former > the latter; Green Star: the former < the latter). c mRNA expression of WNT3A, WNT3, WNT5A, WNT5B, and WNT11 in normal breast, Luminal, Her-2 positive, and TNBC subtypes based on UALCAN (Red p: the former > the latter; Green p: the former < the latter). d mRNA expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure). b Expression of selected canonical Wnt signaling constitutive components in two normal breast cell lines, eight luminal, and seven BLBC cell lines by western blot (S: Short exposure; L: Long exposure).
correlated with basal-like marker KRT5. d CSNK1A1 is positively correlated with EMT-attenuated markers CDH1 and TJFP. e CSNK1A1 is inversely correlated with EMT-activated markers VIM and SNAI. f CSNK1A1 is inversely correlated with WNT5B. g CSNK1A1 is positively correlated with CCND1. (b-g) were based on GEPIA (breast invasive carcinoma; \( n = 1085 \)). h Ultrasound images of BLBC cells bearing mice under B-type ultrasonic imaging system at day 6, 12, and 18 for Bcap-37 following treatment with LGK-974 (5 mg/kg) or pynurin pamoate (1 mg/kg) \( \% \) Percentage of nuclei positive for K67 after 14 days of LGK-974 or pynurin pamoate treatment. Graphs represent mean ± SEM (n = 5 per treatment group; ****p < 0.0001, n.s: no significant). Figure S5. Proposed model of WNT5B governing the phenotypes of BLBC by activating canonical and non-canonical WNT signaling. (PDF 14908 kb)

Abbreviations

AGFR2: Anterior gradient homolog 2; APC: Adenomatous polyposis coli; BLBC: Basal-like breast cancer; CK18: Cytokeratin 18; CK1α: Casein kinase 1α; CK5: Cytokeratin 5; EGFR: Epidermal growth factor receptor; EMT: Epithelial–mesenchymal transition; ER: Estrogen receptor; FoxA1 (also known as TCF7): T Cell Factor 1; TNBC: Triple negative breast cancer; TCF1: Lymphoid Enhancer Factor 1; LRP6: Low-density lipoprotein receptor-related protein 6; PR: Progesterone Receptor; SPARC: Secreted protein acidic and rich in cysteine; TCF7 (also known as TCF7); T Cell Factor 1; TBF: Triple negative breast cancer; TWIST1: Twist family BHLH transcription factor 1; ZEB1 (also known as TCF8); Zinc finger E-box-binding homeobox 1; ZO-1: Zona occludens-1

Acknowledgments

Not applicable.

Authors’ contributions

Project planning was done by XY and SJ, SJ, MZ, and FZ performed the experiments with the help of VY, WZ, TZ, QR, HC, JF, and JS; SI analyzed data, prepared figures and wrote a draft of the paper. XY and SJ conceived the ideas, designed and discussed experiments. XY supervised progress and extensively edited and communicated regarding the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by The Key Program of National Natural Science Foundation of China 81430040 (X.Y.); General Program of National Natural Science Foundation of China 81571738 & 81871403 (J.S.); Medical Science Foundation of China 81571738 & 81871403 (J.S.); The National Key Foundation of China 81430040 (X.Y.); General Program of National Natural Science Foundation of China 81170379, 81671193 and 81871403 (X.Y.); Zhejiang Provincial Key Program of Natural Science Foundation of China Z19A010083; National Key Research and Development Program of China 2016YFA0100900 (J.S.); and Zhejiang Provincial Key Program of Natural Science Foundation of China (2017100003).

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Sir Run Run Shaw Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1Department of Radiology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, Zhejiang, China. 2Department of Orthopaedics, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang, China. 3Department of Pathology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, Zhejiang, China. 4Department of Diagnostic Ultrasound and Echocardiography, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, Zhejiang, China. 5Department of Surgery, Division of Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang, China. 6Key Laboratory of Experimental Animal and Safety Research, Zhejiang Academy of Medical Sciences, Hangzhou 310013, Zhejiang, China. 

Image-Guided Bio-Molecular Intervention Research, Department of Radiology, University of Washington School of Medicine, Seattle, Washington 98109, USA.

Received: 5 June 2019 Accepted: 12 August 2019

Published online: 28 August 2019

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.

2. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406:74–72.

3. Sorlie T, Perou CM, Tibshirani R, Aas T, Geiser S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001;98:10869–74.

4. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A. 2003;100:10994–99.

5. Nielsen TO, Hsu FD, Jensen K, Cheang MC, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10:5367–74.

6. Carey LA, Perou CM, Livasy CA, Dresler LG, Covian D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina breast cancer study. JAMA. 2006;295:2492–502.

7. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol. 2010;28:3271–7.

8. Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, et al. E2f encourages tumorigenic properties with both genetic and snail critical for snail-mediated E-cadherin repression in human breast cancer. J Clin Invest. 2012;122:1469–86.

9. Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miyalya S, et al. Loss of FBPI by snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell. 2013;23:316–31.

10. Luga V, Zhang L, Vitoria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, et al. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell. 2012;151:1542–56.

11. Gujral TS, Chan M, Peshkin L, Sorger PK, Kirschner MW, MacBeath G. A noncanonical Fizzled2 pathway regulates epithelial-mesenchymal transition and metastasis. Cell. 2014;159:44–56.

12. Harper KL, Sosa MS, Engebretsen D, Hosseini H, Cheung JF, Nobre R, et al. Mechanism of early dissemination and metastasis in Her2(+) mammary cancer. Nature. 2016;540(7634):588–92.

13. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in breast cancer. Nat Rev Cancer. 2013;13:111–26.

14. Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330–7.

15. Voloshenko O, Erdmann G, Dubash TD, Augustin I, Metzig M, Moffa G, et al. Wnt secretion is required to maintain high levels of Wnt activity in colon cancer cells. Nat Commun. 2013;4:2610.

16. Sunaga N, Kohno T, Kolligs FT, Fearon ER, Saito R, Yokota J. Constitutive activation of the Wnt signaling pathway by CTNNB1 (beta-catenin) mutations in a subset of human lung adenocarcinoma. Genes Chromosomes Cancer. 2001;31:316–21.

17. Genome Cancer Research N, Kandzuh C, Schultz N, Cherniack AD, Aksani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497:673–7.

18. Riviera MN, Kim WJ, Wells J, Dricoll DR, Brannigan BW, Han M, et al. An X chromosome gene, WTX, is commonly inactivated in Wilms tumor. Science. 2007;315:642–5.

19. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, et al. Beta-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol. 2012;25:209–31.

20. Semenov MV, Habas R, Macdonald BT, He X. SnapShot: noncanonical Wnt signaling pathways. Cell. 2007;131:1378.

21. Shi J, Wang Y, Zeng L, Wu Y, Deng J, Zhang Q, et al. Disrupting the interaction of BRD4 with diacylated twist suppresses tumorigenesis in basal-like breast cancer. Cancer Cell. 2014;25:210–25.
22. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fev T, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell. 2006;10:515–27.

23. Ji J, Weng Q, Zhang F, Xiong F, Jin Y, Hui J, et al. Non-small-cell lung cancer: feasibility of Intratumoral radiofrequency hyperthermia-enhanced herpes simplex virus thymidine kinase gene therapy. Radiology. 2018;288:612–20.

24. Jezquel P, Campone M, Gouraud W, Guerin-Charbonnel C, Leuc C, Ricolleau G, et al. bc-GeneXminer: an easy-to-use online platform for gene prognostic analyses in breast cancer. Cancer Res Treat Rev. 2012;131:765–75.

25. Jezquel P, Frenel JS, Campion L, Guerin-Charbonnel C, Gouraud W, Ricolleau G, et al. bc-GeneXminer 3.0: new mining module computes breast cancer gene expression correlation analyses. Database (Oxford). 2013;2013:bao060.

26. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponec-Rodriguez J, Chakravarti B, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19:649–58.

27. Cemal E, Gao J, Dogruoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401–4.

28. Gao J, Aksylo-BrunA, Dogruoz U, Desideri G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.

29. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards a knowledge-based human protein atlas. Nat Biotechnol. 2010;28:1248–50.

30. Consortium GT, Laboratory DA, Coordinating Center -Analysis Working G, Statistical Methods groups-Analysis Working G, Enhancing Gg, Fund NHIC, et al. Genetic effects on gene expression across human tissues. Nature. 2017;550:204–13.

31. Gyorffy B, Lanczycki I, Elkdoun AC, Denkert C, Bucdeszies J, Li Q, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat. 2010;123:725–31.

32. Barretina J, Caponigro G, Skranysz V, Venkatesan K, Margolin AA, Kim S, et al. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483:603–7.

33. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W908-W102.

34. Soklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45:D362-D8.

35. Jiang X, Hao HK, Crowney JD, Woolfenden S, Bottiglio C, Ng N, et al. Inactivating mutations of RNF43 confer Wnt dependency in pancreatic cancer genomics data. Cancer Discov. 2012;2:1248–58.

36. Jezequel P, Frenel JS, Campion L, Guerin-Charbonnel C, Gouraud W, Ricolleau G, et al. bc-GeneXminer 3.0: new mining module computes breast cancer gene expression correlation analyses. Database (Oxford). 2013;2013:bao060.

37. Jiang S, Zhang M, Sun J, Yang X. Casein kinase 1alpha: biological mechanisms and theranostic potential. Cell Commun Signal. 2018;16:23.

38. Li B, Fei DL, Flaveny CA, Dahmane N, Baubet V, Wang Z, et al. Pyrvinium attenuates hedgehog signaling downstream of smoothened. Cancer Res. 2014;74:4811–21.

39. Deng L, Lei Y, Liu R, Li J, Yuan K, Li Y, et al. Pyrvinium targets autophagy addiction to promote cancer cell death. Cell Death Dis. 2013;4:e614.

40. Xu W, Lacerda L, Debeuk BG, Atkinson RL, Solley TN, Li L, et al. The anthelmintic drug pyrvinium pamoate targets aggressive breast cancer. PLoS One. 2013;8:e71508.

41. Xu L, Zhang L, Hu C, Liang S, Fei X, Yan N, et al. WNT pathway inhibitor pyrvinium pamoate inhibits the self-renewal and metastasis of breast cancer stem cells. Int J Oncol. 2014;60:1175–86.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.