The clinicopathological significance of ubiquitin-conjugating enzyme E2C, leucine-rich repeated-containing G protein-coupled receptor, WW domain-containing oxidoreductase, and vasculogenic mimicry in invasive breast carcinoma

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Abstract
Ubiquitin-conjugating enzyme E2C (UBE2C), a crucial part of the ubiquitin—conjugating enzyme complex, is reported to promote progression of various cancers. Leucine-rich repeated-containing G protein-coupled receptor (LGR5), a biomarker of cancer stem cells, is reported to be responsible for the initiation and progression of cancers. WW domain-containing oxidoreductase (WWOX), a suppressor of tumor, is reported to inhibit initiation and progression of cancers. Vasculogenic mimicry (VM), a new blood supply pattern, is associated with progression of cancers. However, the clinicopathological significance of UBE2C, LGR5, WWOX, and VM in invasive breast carcinoma (IBC) remains elusive. The aim of this study is to investigate the positive rate of UBE2C, LGR5, WWOX, and VM in IBC and their clinical significance.

Positive rates of UBE2C, LGR5, WWOX, and VM in 247 whole IBC samples were detected through immunohistochemistry. Patients data (including clinical, demography, follow-up) were collected.

Levels of UBE2C, LGR5, VM, and microvessel density (MVD) were significantly higher, and level of WWOX was significantly lower in IBC specimens when compared with normal mammary gland tissues. Levels of UBE2C, LGR5, VM, and MVD were all positively associated with tumor stages, lymph node metastasis (LNM) stages, tumor grades, and tumor-node-metastasis (TNM) stages, and unfavorably with patients’ overall survival (OS) and disease-free survival (DFS). Level of WWOX was negatively associated with tumor stages, LNM stages, grades, and TNM stages, and favorably with patients’ OS and DFS. Multivariate analysis indicated that levels of UBE2C, LGR5, VM, MVD, and WWOX, as well as TNM stages were independently prognostic factors for OS and DFS in patients with IBC.

UBE2C, LGR5, VM, MVD, and WWOX may be considered as promising indicators of IBC prognosis.

Abbreviations: AJCC = American Joint Committee on Cancer, CSCs = cancer stem cells, DFS = disease-free survival, ER = estrogen receptor, HER2 = human epithelial growth factor receptor 2, HPF = high-power-field, IBC = invasive breast carcinoma, LGR5 = leucine-rich repeated-containing G protein-coupled receptor, LNM = lymph node metastasis, MVD = microvessel density, OS = overall survival, PR = progesterone receptor, TNM = tumor node metastasis, UBE2C = ubiquitin-conjugating enzyme E2C, VM = vasculogenic mimicry, WHO = World Health Organization, WWOX = WW domain-containing oxidoreductase.

Keywords: invasive breast carcinoma, leucine-rich repeated-containing G protein-coupled receptor, microvessel density, ubiquitin-conjugating enzyme E2C, vasculogenic mimicry, WW domain-containing oxidoreductase
1. Introduction

Breast carcinoma was the most common diagnosed cancer among worldwide women and was estimated 520,000 deaths in the worldwide in 2012.\textsuperscript{1,2} Breast cancer is a highly heterogeneous disease which makes it urgent to identify some biomarkers for early diagnosis, progression and prognosis judgement, and treatment. Indeed, estrogen receptor (ER), progesterone receptor (PR), and human epithelial growth factor receptor (HER2) amplification are related to distinct molecular subtypes and prognostic and therapeutic values.

Ubiquitin-conjugating enzyme E2C (UBE2C), also named as UbcH10, is a member of the E2 gene family and plays an important role in mitotic cyclin degradation and cell cycle progression.\textsuperscript{2} UBE2C gene is located on chromosome 20q13 and encodes a 19.65kDa protein. In normal tissues, UBE2C is almost undetectable,\textsuperscript{3} whereas it overexpresses in many cancers.\textsuperscript{4} The previous studies have demonstrated that UBE2C is involved in many biological behaviors, such as tumorigenesis, proliferation, cell cycle, and apoptosis.\textsuperscript{5-8}

Tumor recurrence and metastasis should be related to a subpopulation of cancer cells which names cancer stem cells (CSCs). Accumulating evidence has demonstrated that CSCs have the capacity to self-renew, multi-directional differentiate, and are responsible for the resistance of chemo- or radio-therapy. The leucine-rich repeat-containing G-protein-coupled receptors 5 (LGR5) is a biomarker of stem cells in many organs, such as intestine, hair follicle, stomach, mammary gland, and ovary.\textsuperscript{9-13} LGR5, which was originally considered as a Wnt/Tcf4 target gene, is a member of glycoprotein hormone receptor family.\textsuperscript{14} LGR5 is consisted of a large leucine-rich domain and N terminal of the peptide. Overexpression of LGR5 can promote cancer cells proliferation, progression, metastasis, and CSCs maintenance.\textsuperscript{14,15}

WW domain-containing oxidoreductase (WWOX) is considered as a suppressor and resides in one of the most active common fragile sites which named FRA16D.\textsuperscript{16} WWOX, which encodes a 19.65kDa protein. In normal tissues, UBE2C is almost undetectable,\textsuperscript{3} whereas it overexpresses in many cancers.\textsuperscript{4} The previous studies have demonstrated that UBE2C, LGR5, WWOX, MVD, and VM. The purpose of this study is to examine the hypothesis that these biomarkers should be mutually associated and be associated with progression and prognosis in invasive breast carcinoma (IBC).

2. Methods

2.1. Patients and specimens

Two hundred forty-seven patients (median age: 54.7 years; range: 26–77 years) who were diagnosed IBC at the Department of Pathology of our hospital were collected, from January 2012 to December 2013, along with the corresponding adjacent normal mammary tissues (5 cm away from the tumor edges). Patients who had undergone any anti-cancer therapy were excluded. The clinicopathological characteristics of the 247 IBC tissue specimens were shown in Table 1. Patients’ follow-up data was also

| Table 1 Patients characteristic | Frequency (n) | Percentage (%) |
|--------------------------------|--------------|----------------|
| Age, y                        |              |                |
| < 50                          | 112          | 45.3           |
| > 50                          | 135          | 54.7           |
| Smoking                       |              |                |
| No                            | 203          | 82.2           |
| Yes                           | 44           | 17.8           |
| Alcohol                       |              |                |
| No                            | 187          | 75.7           |
| Yes                           | 60           | 24.3           |
| Location                      |              |                |
| Left                          | 123          | 49.8           |
| Right                         | 115          | 46.6           |
| Both                          | 9            | 3.6            |
| Size, cm                      |              |                |
| ≤ 2.0                         | 73           | 29.6           |
| > 2.0, ≤ 5.0                  | 145          | 58.7           |
| > 5.0                         | 29           | 11.7           |
| Grade                         |              |                |
| Well                          | 58           | 23.5           |
| Moderate                      | 119          | 48.2           |
| Poor                          | 70           | 28.3           |
| Tumor stages                  |              |                |
| T1                            | 78           | 31.6           |
| T2                            | 134          | 54.3           |
| T3                            | 24           | 9.7            |
| T4                            | 11           | 4.5            |
| Lymph node metastasis stages  |              |                |
| N0                            | 129          | 52.2           |
| N1                            | 78           | 31.6           |
| N2                            | 35           | 14.2           |
| N3                            | 5            | 2.0            |
| TNM stage                     |              |                |
| I                             | 42           | 17.0           |
| II                            | 149          | 60.3           |
| III                           | 56           | 22.7           |
| ER expression                 |              |                |
| Negative                      | 113          | 45.7           |
| Positive                      | 134          | 54.3           |
| PR expression                 |              |                |
| Negative                      | 128          | 51.8           |
| Positive                      | 119          | 48.2           |
| HER2 expression               |              |                |
| Negative                      | 172          | 69.6           |
| Positive                      | 75           | 30.4           |

ER=estrogen receptor, HER2=human epithelial growth factor receptor 2, PR=progesterone receptor, TNM=tumor node metastasis.
collected (at 3-month intervals through mobile phone and social applications). Overall survival (OS) time was calculated from surgery date to December 2017 or her death date (mean OS: 55.7 months; range: 10–83 months). Tumor stages and TNM stages both were evaluated in accordance with the 8th edition of the guidelines issued by American Joint Committee on Cancer (AJCC). Grades of differentiation were evaluated in accordance with the guidelines issued by World Health Organization (WHO).

2.2. Reagent and immunohistochemistry

Mouse anti-human monoclonal antibody against UBE2C (1F5D3) and CD34 (ab54028), and rabbit anti-human polyclonal antibody against LGR5 (ab75732) and WWox (ab74091) were purchased from the Abcam, Co., Ltd., Cambridge, Massachusetts, UK. Rabbit anti-human monoclonal antibody against human epidermal growth factor receptor 2 (HER2, EP3), estrogen receptor (ER, SP1), and progesterone receptor (PR, SP2) and other reagents were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, Fujian, China. All specimens were fixed in 10% neutral formalin solution and embedded in paraffin. Continuous 4-μm-thick sections were cut. Immunohistochemistry was performed following the Elivision Plus method, and the procedure was performed following the kit instructions. All sections were deparaffinized and dehydrated using routine methods. Citrate buffer solution was used antigen repair, and endogenous peroxidase activity was quenched by methanol containing 3% H2O2 solution. Then were blocked with goat serum. UBE2C, LGR5, CD34, WWox, HER2, ER, and PR primary antibodies were added, subsequently, all sections were incubated at 4°C overnight. Then reagent A and reagent B were added. All sections were performed periodic acid-Schiff (PAS)-CD34 dual staining. All sections were developed in diaminobenzidine (DAB) substrate. Finally, sections were re-dyed with hematoxylin.

2.3. Assessment of immunohistochemistry

Ten randomly selected high-power-field (HPF) fields of every IBC section were to avoid potential intratumoral heterogeneity of any biomarker expression. The intensity of immunostaining was scored as follows: no staining was 0; weak staining was 1; moderate staining was 2; strong staining was 3. The percentage of positive cells was scored as follows: <11% was 1; 11% to 50% was 2; 51% to 75% was 3; >75% was 4. The final scores were yielded by multiplying the intensity score and the extent score (range 0–12). The eventual determination of the results was considered as positive (score ≥2). In accordance with 2013 ASCO/CAP guidelines, HER2 expression in 10% of cancer cells was considered as positive. ER and PR expression in no <1% of cancer cells were considered as positive. If there was difference between assessment results from the 2 independent pathologists, the results were reassessed. MVD was determined by the mean number of vessels as well as vessel-like (VM) tubes. The method was adopted from Yue and Chen with some modifications.

2.4. Statistical analysis

Chi-square tests were used to assess the positive rates of UBE2C, LGR5, WWox, MVD, and VM in IBC and the control tissues as well as the associations between these biomarkers expression and the clinicopathological characteristics of IBC. Correlation analysis was carried out by using Spearman correlation test. Univariate OS and DFS analyzes were performed using the Kaplan–Meier method with log-rank tests. Multivariate OS and DFS analyzes were performed using Cox regression model tests. P < .05 was defined statistically significance. All data of statistical analyzes were using SPSS 19.0 software (Chicago, IL).

3. Results

3.1. The positive rates of UBE2C, LGR5, and VM were significantly higher in IBC tissues than those in the control tissues, inversely to them, WWox expression was significantly lower in IBC tissues

The positive expression of UBE2C was mainly confined nuclei and cytoplasm; the positive expression of LGR5 was mainly confined cytoplasm and membrane; the positive expression of WWox was mainly confined cytoplasm. The positive rate of UBE2C expression in IBC (58.7%, 145/247) was significantly higher than that in the control group (0%, 0/247; P < .001; Fig. 1A and B). The positive rate of LGR5 expression in IBC (61.5%, 152/247) was significantly higher than that in the control group (8.9%, 22/247; P < .001; Fig. 1C and D). The positive rate of WWox expression in IBC (47.0%, 116/247) was significantly lower than that in the control group (87.0%, 215/247; P < .001; Fig. 1C and D). The positive rate of VM in IBC (32%, 79/247) was signiﬁcantly higher than that in the control group (0%, 0/247; P < .001; Fig. 1E and F).

3.2. The positive rates of UBE2C, LGR5, and VM were positively related to tumor size, histological grades, tumor stages, LNM stages as well as TNM stages, inversely to them, WWox expression is negatively related to these clinicopathological characteristics

UBE2C expression in IBC was positively related to alcohol, tumor size, histological grades, tumor stages, LNM stages, and TNM stages. LGR5 expression in IBC was positively related to tumor size, histological grades, tumor stages, LNM stages as well as TNM stages. WWox expression in IBC was negatively related to tumor size, histological grades, tumor stages, LNM stages, and TNM stages. The positive rate of VM in IBC was positively related to tumor size, histological grades, tumor stages, LNM stages, and TNM stages. The score of MVD in IBC was positively related to histological grades and TNM stages (Table 2).

3.3. Spearman correlation test

Correlation analysis revealed that the positive rate of WWox in IBC was negatively correlated with the positive rate of UBE2C (r = -0.512, P < .001), LGR5 (r = -0.473, P < .001), VM (r = -0.210, P = .001), MVD (r = -0.199, P = .002), and HER2 (r = -0.410, P < .001), and positively correlated with ER expression (r = 0.262, P < .001). The positive rate of UBE2C in IBC was positively associated with the positive rate of LGR5 (r = 0.436, P < .001), VM (r = 0.258, P < .001), MVD (r = 0.135, P = .034), HER2 (r = 0.268, P < .001) and negatively associated with ER expression (r = -0.193, P = .002). The positive rate of LGR5 in IBC was positively associated with VM (r = 0.167, P = .008),
MVD \( (r = 0.145, P = .023) \), HER2 \( (r = 0.251, P < .001) \), and negatively associated with ER expression \( (r = -0.138, P = .013) \). The positive rate of VM in IBC was positively associated with MVD \( (r = 0.284, P < .001) \), HER2 \( (r = 0.227, P < .001) \), and negatively associated with PR expression \( (r = -0.192, P = .002) \). The score of MVD in IBC was positively associated with HER2 \( (r = 0.165, P = .010) \) (Table 3).

3.4. Univariate and multivariate analyzes

As shown in Fig. 2A, univariate OS analysis suggested that OS time of UBE2C+ for patients with IBC were significantly longer than that of UBE2C− for patients \( (log-rank = 56.737, P < .001) \). As shown in Fig. 2B, the univariate OS time of LGR5+ patients were significantly lower than in LGR5− patients \( (log-rank = 60.951, P < .001) \). As shown in Fig. 2C, the univariate OS time of WWOX+ patients were significantly longer than in WWOX− patients \( (log-rank = 80.033, P < .001) \). As shown in Fig. 2D, the univariate OS time of VM+ patients were significantly shorter than in VM− patients \( (log-rank = 34.773, P < .001) \). As shown in Fig. 2E, the univariate OS time of MVD\textsuperscript{low} score patients were significantly lower than in MVD\textsuperscript{low score} patients \( (log-rank = 22.534, P < .001) \). As shown in Fig. 2F and G, the univariate OS time of ER+ or PR+ patients were significantly longer than in ER− or PR− patients \( (log-rank = 18.999, P < .001); log-rank = 11.569, P = .001 \), respectively). As shown in Fig. 2H, the univariate OS time of HER2+ patients were significantly lower than in HER2− patients \( (log-rank = 37.689, P < .001) \) (Table 4). As shown in Fig. 3A, B, D, E, H, the univariate DFS time of UBE2C+, or LGR5+, or VM+, or MVD\textsuperscript{high} score, or HER2+ patients were significantly longer than in UBE2C−, or LGR5−, or VM−, or MVD\textsuperscript{low} score, or HER2− patients \( (log-rank = 58.314, P < .0001); log-rank = 59.612, P < .001); log-rank = 36.745, P < .001; log-rank = 21.976, P < .001); log-rank = 41.686, P < .001 \), respectively). As shown in Fig. 3C, G, F, the univariate DFS time of WWOX+, or ER+, or PR+ patients \( (log-rank = 20.135, P < .001); log-rank = 13.735, P < .001 \), respectively) (Table 5).

Multivariate analysis of OS suggested that positive expression of UBE2C, LGR5, WWOX, ER and VM, MVD as well as TNM stages were independent factors affecting patients’ OS (Table 6). Multivariate analysis of DFS suggested that positive expression of UBE2C, LGR5, WWOX, ER and VM, MVD, and TNM stages were independent factors affecting patients DFS (Table 7).

4. Discussion

Breast cancer is one of the most common malignant tumors of women. Invasive breast carcinoma (IBC) is a highly heterogenous disease that leads to a serious threat to women’s health and lives. It is urgent to investigate the pathogenesis of IBC and comprehensively evaluate biomarkers for IBC. UBE2C is considered to play an important role in the ubiquitin proteasome proteolytic pathway which is considered to be associated with occurrence and progression of tumors.\textsuperscript{[23]} In this study, expression of UBE2C was significantly higher in IBC tissues than that in the normal mammary tissues, and its overexpression was positively associated with alcohol, tumor size, histological grades, tumor stages, LNM stages, and TNM stages. In addition, Kaplan–Meier survival analysis showed that IBC patients with UBE2C+ had significantly lower OS or DFS time than did UBE2C− patients. These results suggested that UBE2C overexpression promoted IBC progression and metastasis, as well as played a potential prognostic indicator for IBC.

LGR5, also named GPR49, is a common biomarker of CSCs. LGR5 knockdown CSCs showed lower capacity of proliferation and sphere formation.\textsuperscript{[30,31]} In the present study, the data indicated that LGR5 expression was significantly related to tumor size, histological grades, tumor stages, LNM stages as well as TNM stages, similar to those reported previously studies.\textsuperscript{[32,33]} These findings confirmed that LGR5 expression should play an important role in progression and metastasis of IBC. Moreover, the data showed that LGR5+ patients had lower OS or DFS time.
than LGR5 patients. LGR5 should be considered a useful biomarker for predicting prognosis of IBC.

WWOX is a suppressor gene and suppresses tumorigenesis through promoting apoptosis and maintaining genome integrity.[34,35] In our study, the data showed that WWOX expression was inversely related to tumor size, histological grades, tumor stages, LNM stages as well as TNM stages, parallel to those reported previously studies.[36,37] These results confirmed that loss of WWOX should promote invasion and metastasis of IBC. Furthermore, the data also confirmed that WWOX+ patients had longer OS or DFS time than WWOX– patients. Positive expression of WWOX should mean a favorable prognosis for IBC patients.

Several studies have demonstrated that angiogenesis promotes tumor cells proliferation, invasion, and metastasis in many human cancers. It is well known that anti-angiogenic therapy is a highlight method for anti-cancer therapy. However, it is still unsatisfactory for the benefit of anti-angiogenic therapy. VM is a channel which is lining cancer cells may partly explain this unsatisfactory benefit. In this study, the data showed that VM+ or MVDhigh score was significantly associated with histological grades and TNM stages, similar to those reported previously.

Table 2
The association between UBE2C, LGR5, WWOX, VM, MVD, and clinicopathological characteristics.

| Characteristics | UBE2C | LGR5 | WWOX | VM | MVD |
|-----------------|-------|------|------|----|-----|
| Age, y          |       |      |      |    |     |
| ≤50             | 44    | 68   | 0.59 | .808 | 0.239 | 0.090 | 0.222 |
| >50             | 58    | 77   | 0.19 | 0.180 | 0.059 | 0.702 | 0.487 |
| Smoking         |       |      |      |    |     |
| No              | 88    | 115  | 0.01 | 0.124 | 0.952 | 0.081 |
| Yes             | 14    | 30   | 0.23 | 0.37  | 0.419 | 0.24  | 0.36 |
| Alcohol         |       |      |      |    |     |
| No              | 84    | 103  | 0.04 | 0.981 | 0.124 | 0.952 | 0.081 |
| Yes             | 18    | 42   | 0.23 | 0.41  | 0.419 | 0.24  | 0.36 |
| Location        |       |      |      |    |     |
| Left            | 56    | 67   | 0.303| 0.387 | 0.963 | 0.795 | 0.480 |
| Right           | 43    | 72   | 0.303| 0.387 | 0.963 | 0.795 | 0.480 |
| Size, cm        |       |      |      |    |     |
| ≤2.0            | 46    | 27   | 0.01 | 0.001 | 0.001 | 0.021 | 0.610 |
| >2.0, ≤5.0      | 52    | 93   | 0.25 | 0.001 | 0.001 | 0.001 | 0.001 |
| >5.0            | 4     | 25   | 0.54 | 0.32  | 0.44  | 0.47  | 0.72 |
| Grade           |       |      |      |    |     |
| Well            | 45    | 13   | 0.01 | 0.001 | 0.001 | 0.012 | 0.721 |
| Moderate        | 50    | 69   | 0.01 | 0.001 | 0.001 | 0.012 | 0.721 |
| Poor            | 7     | 63   | 0.01 | 0.001 | 0.001 | 0.012 | 0.721 |
| Tumor stages    |       |      |      |    |     |
| T1              | 51    | 27   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| T2              | 45    | 89   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| T3              | 4     | 20   | 0.54 | 0.32  | 0.44  | 0.47  | 0.72 |
| T4              | 2     | 9    | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| LNM stages      |       |      |      |    |     |
| N0              | 70    | 59   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| N1              | 25    | 53   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| N2              | 7     | 28   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| N3              | 0     | 5    | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| TNM stage       |       |      |      |    |     |
| I               | 33    | 9    | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| II              | 59    | 90   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| III             | 10    | 46   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| ER              |       |      |      |    |     |
| Negative        | 35    | 78   | 0.002| 0.013 | 0.001 | 0.001 | 0.001 |
| Positive        | 67    | 67   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| PR              |       |      |      |    |     |
| Negative        | 49    | 79   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| Positive        | 53    | 66   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| HER2            |       |      |      |    |     |
| Negative        | 86    | 86   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |
| Positive        | 16    | 59   | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 |

Because the mean score of MVD is 20.8, MVD ≤21 is low group, MVD >21 is high group. ER = estrogen receptor, HER2 = human epithelial growth factor receptor 2, LGR5 = leucine-rich repeated-containing G protein-coupled receptor, LNM = lymph node metastasis, MVD = microvessel density, PR = progesterone receptor, TNM = tumor node metastasis, UBE2C = ubiquitin-conjugating enzyme E2C, VM = vasculogenic mimicry, WWOX = WW domain-containing oxidoreductase.

*Is negative association.  †Is positive association.
Kaplan–Meier analysis also indicated that VM+ or MVDhigh score patients had an unfavorable OS or DFS time than VM− or MVDlow score patients. VM and MVD are also considered a useful potential indicator for prediction of IBC.

In this study, our data also demonstrated that expression of UBE2C, LGR5, and WWOX and VM, MVD, as well as TNM stages were independent prognostic factors of OS or DFS for patients with IBC. In addition, our data also indicated that WWOX expression was inversely associated with UBE2C, LGR5, VM, and MVD score; UBE2C, LGR5, VM, and MVD are positively associated with each other. The origin of breast cancer, in some studies, is considered to derive from putative CSCs.[9] It is believed that CSCs can promote the malignant transformation of cells in part by activation of Wnt/β-catenin signal pathway.[32] Overexpression of LGR5 is thought to promote breast cancer progression and CSCs maintenance.[14]

| Variable | UBE2C | LGR5 | WWOX | VM |
|----------|-------|------|-------|-----|
|          | −     | +    | −     | +   |
|          | −     | 65   | 37    | 23  | 79  | 84  | 18  |
|          | +     | 30   | 115   | 108 | 37  | 84  | 61  |
|          | −     | 0.436| <.001 |   | −0.512| <.001|   | 0.258| <.001 |
|          | +     | 23   | 79    |   | 84  | 18  |   | 0.167| .008 |
| LGR5     | −     | 37   | 115   | 22 | 73  | 74  | 21  |
|          | +     | 109  | 43    |   | 94  | 58  |   |   |   |
|          | −     | −0.473| <.001 |   | −0.473| <.001|   | −0.210| .001 |
|          | +     | 77   | 54    |   | 91  | 25  |   |   |   |
| WWOX     | −     | 23   | 108   | 22 | 109 | 77  | 54  |
|          | +     | 73   | 43    |   | 91  | 25  |   |   |   |
|          | −     | 0.135| .034  |   | 0.145| .023|   | 0.199| .002 |
|          | +     | 56   | 67    |   | 53  | 70  |   | 0.284| <.001 |
| MVD      | Low   | 59   | 64    | 56 | 67  | 53  | 70  |
|          | High  | 43   | 81    | 39 | 85  | 78  | 46  |
|          | −     | 1.058| <.001 |   | 0.167| .008|   | −0.210| .001 |
|          | +     | 84   | 84    |   | 77  | 91  |   |   |   |
|          | −     | 43   | 81    |   | 68  | 56  |   |   |   |
|          | +     | 18   | 61    |   | 54  | 25  |   |   |   |

UBE2C = ubiquitin-conjugating enzyme E2C, LGR5 = leucine-rich repeated-containing G protein-coupled receptor, MVD = microvessel density, VM = vasculogenic mimicry, WWOX = WW domain-containing oxidoreductase.

|      |      |      |      |      |
|------|------|------|------|
|      |      |      |      |
| LGR5 = leucine-rich repeated-containing G protein-coupled receptor, MVD = microvessel density, UBE2C = ubiquitin-conjugating enzyme E2C, VM = vasculogenic mimicry, WWOX = WW domain-containing oxidoreductase.

In Figure 2, Kaplan–Meier analysis of overall survival time of patients with invasive breast carcinoma. A: Overall survival of all patients in relation to UBE2C expression (log-rank = 56.737, P < .001); B: OS of all patients in relation to LGR5 (log-rank = 60.951, P < .001); C: OS of all patients in relation to WWOX expression (log-rank = 80.033, P < .001); D: OS of all patients in relation to VM (log-rank = 34.773, P < .001); E: OS of all patients in relation to MVD (log-rank = 22.534, P < .001); F: OS of all patients in relation to ER (log-rank = 18.999, P < .001); G: OS of all patients in relation to PR (log-rank = 11.569, P = .001); H: OS of all patients in relation to HER2 (log-rank = 37.689, P < .001). In A, B, C, D, E, F, G, and H analyses, the green line represents positive staining of factors (MVD score ≥21 is positive) and the blue line represents negative staining factors (MVD score <21 is negative). LGR5 = leucine-rich repeated-containing G protein-coupled receptor, MVD = microvessel density, OS = overall survival, PR = progesterone receptor, UBE2C = ubiquitin-conjugating enzyme E2C, VM = vasculogenic mimicry.
### Table 4
Results of univariate analyses of overall survival (OS) time.

| Variable | n  | Mean OS (mo) | Log-rank | P value |
|----------|----|--------------|----------|---------|
| UBE2C    |    |              | 56.737   | <.001   |
| Negative | 102| 67.0±11.6    |          |         |
| Positive | 145| 47.8±15.8    |          |         |
| LGR5     |    |              | 60.951   | <.001   |
| Negative | 95 | 67.2±12.8    |          |         |
| Positive | 152| 48.6±15.4    |          |         |
| WWOX     |    |              | 80.033   | <.001   |
| Negative | 131| 45.7±14.7    |          |         |
| Positive | 116| 67.1±11.5    |          |         |
| VM       |    |              | 34.773   | <.001   |
| Negative | 168| 60.5±15.5    |          |         |
| Positive | 79 | 45.7±15.8    |          |         |
| MVD      |    |              | 22.534   | <.001   |
| Low      | 123| 60.5±16.2    |          |         |
| High     | 124| 51.1±16.7    |          |         |
| ER       |    |              | 18.999   | <.001   |
| Negative | 113| 48.7±17.7    |          |         |
| Positive | 134| 61.7±14.0    |          |         |
| PR       |    |              | 11.569   | .001    |
| Negative | 128| 52.8±16.7    |          |         |
| Positive | 119| 59.0±16.9    |          |         |
| HER2     |    |              | 37.689   | <.001   |
| Negative | 172| 60.9±14.7    |          |         |
| Positive | 75 | 43.1±11.5    |          |         |

**ER** = estrogen receptor, **HER2** = human epithelial growth factor receptor 2, **LGR5** = leucine-rich repeated-containing G protein-coupled receptor, **MVD** = microvessel density, **PR** = progesterone receptor, **UBE2C** = ubiquitin-conjugating enzyme E2C, **VM** = vasculogenic mimicry, **WWOX** = WW domain-containing oxidoreductase.

### Table 5
Results of univariate analyses of disease-free survival (DFS) time.

| Variable | N  | Mean DFS (mo) | Log-rank | P value |
|----------|----|---------------|----------|---------|
| UBE2C    |    |              | 58.314   | <.001   |
| Negative | 102| 62.1±12.3    |          |         |
| Positive | 145| 43.3±15.0    |          |         |
| LGR5     |    |              | 59.612   | <.001   |
| Negative | 95 | 62.5±13.0    |          |         |
| Positive | 152| 43.9±14.7    |          |         |
| WWOX     |    |              | 82.818   | <.001   |
| Negative | 131| 41.2±13.8    |          |         |
| Positive | 116| 62.2±12.1    |          |         |
| VM       |    |              | 36.745   | <.001   |
| Negative | 168| 55.7±15.5    |          |         |
| Positive | 79 | 41.2±114.9   |          |         |
| MVD      |    |              | 21.976   | <.001   |
| Low      | 123| 55.9±16.0    |          |         |
| High     | 124| 46.2±16.1    |          |         |
| ER       |    |              | 20.135   | <.001   |
| Negative | 113| 44.1±16.7    |          |         |
| Positive | 134| 56.9±14.4    |          |         |
| PR       |    |              | 13.735   | <.001   |
| Negative | 128| 48.0±16.0    |          |         |
| Positive | 119| 54.3±17.0    |          |         |
| HER2     |    |              | 41.686   | <.001   |
| Negative | 172| 56.0±14.8    |          |         |
| Positive | 75 | 40.0±15.3    |          |         |

**ER** = estrogen receptor, **HER2** = human epithelial growth factor receptor 2, **LGR5** = leucine-rich repeated-containing G protein-coupled receptor, **MVD** = microvessel density, **PR** = progesterone receptor, **UBE2C** = ubiquitin-conjugating enzyme E2C, **VM** = vasculogenic mimicry, **WWOX** = WW domain-containing oxidoreductase.

![Figure 3](image-url)

Figure 3. Kaplan–Meier analysis of disease-free survival time of patients with invasive breast carcinoma. A: Disease-free survival of all patients in relation to UBE2C expression (log-rank = 58.314, P < .0001); B: DFS of all patients in relation to LGARS (log-rank = 59.612, P < .001); C: DFS of all patients in relation to WWOX expression (log-rank = 82.818, P < .001); D: DFS of all patients in relation to VM (log-rank = 36.745, P < .001); E: DFS of all patients in relation to MVD (log-rank = 21.976, P < .001); F: DFS of all patients in relation to ER (log-rank = 20.135, P < .001); G: DFS of all patients in relation to PR (log-rank = 13.735, P < .001); H: DFS of all patients in relation to HER2 (log-rank = 41.686, P < .001). In A, B, C, D, E, F, G, and H analyses, the green line represents positive staining of factors (MVD score ≥ 21 is positive) and the blue line represents negative staining factors (MVD score < 21 is negative). DFS = disease-free survival, HER2 = human epithelial growth factor receptor 2, LGR5 = leucine-rich repeated-containing G protein-coupled receptor, MVD = microvessel density, PR = progesterone receptor, UBE2C = ubiquitin-conjugating enzyme E2C, VM = vasculogenic mimicry.
UBE2C promotes degradation of mitotic cyclins and cell cycle progression and regulates anaphase-promoting complex.\cite{137} Overexpression of UBE2C leads to chromosome missegregation and changes the cell cycle profile, therefore, promoting cell proliferation.\cite{138} The microenvironment where CSCs reside are mainly composed of microvessel and microlymphatic vessels. It is reported that CSCs are able to differentiate tumor cells, endothelial cells, and other cells.\cite{139,40} So, CSCs are able to mimic endothelial cells to form VM and differentiate endothelial cells to form vessel in order to meet tumor growth and invasiveness. In the meanwhile, loss of heterozygosity and hypermethylation of WWOX also promotes breast tumorigenesis and further facilitates cancer progression, and induces angiogenesis.\cite{177–199} Overall, our findings confirmed that there is a complex relationship between the above biomarkers and IBC progression and prognosis. Combined investigation of these biomarkers, to a certain extent, the interaction of these biomarkers should be considered to reflect the progression and prognosis of IBC cells, so providing a potential choice of therapeutic target. The present study has already drawn out some conclusions, however, the size of samples in our study is relatively small and experimental method is relatively simple. The further studies with larger sized samples, such as in vitro, in vivo, and molecular experiment, are needed to support the present observations.

### 5. Conclusions

Our study demonstrated that UBE2C, LGR5, WWOX, VM, and MVD are associated with time of OS or DFS among patients with IBC. Therefore, UBE2C, LGR5, WWOX, VM, and MVD should be considered as useful and biomarkers in IBC, as well as potential targets for IBC.

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### Author contributions

Miao Chen and Rong Shen carried out the design, analysis of pathology, and drafted the manuscript. Ting Wu and Pan Huang carried out sample collection and coordination. Qixiang Shao performed the immunohistochemical staining. All authors read and approved the manuscript.

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