An Association Study Between VDR and HER2 in Breast Cancers Reveals the Potential of Combined Diagnosis and Prognosis

Aimei Dai (✉ daiaimei@mail2.sysu.edu.cn)

Sun Yat-sen University School of Life Science

Ruilin Han

SYSU: Sun Yat-Sen University

Dongjing Chen

SYSU: Sun Yat-Sen University

Research

Keywords: Vitamin D receptor (VDR), human epidermal growth factor receptor 2 (HER2), combined diagnosis, survival rates, Breast cancer

Posted Date: October 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-940192/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Breast cancer is generally acknowledged as a heterogeneous disease, with complete characterization and underlying technologies available to predict outcomes. Biomarkers, especially the human epidermal growth factor receptor 2 (HER2), are keys to characterize breast cancer and optimize the patients' clinical outcomes. Vitamin D has an anti-cancer effect in breast cancer, which is related to the expression level of the vitamin D receptor (VDR). Despite these remarkable achievements, there is limited focus on the combined effects of VDR and HER2, which may provide specific diagnostic and prognostic potential in breast cancers.

Results: In this study, we evaluated the association between VDR and HER2-status-related clinical prognostic characters by processing the breast cancer data of 2433 cases obtained from *cBioPortal*, discovering a high and positive correlation between VDR and HER2 in breast cancer. We also found substantial genes that are significantly correlated with both VDR and HER2 expressions. Those negatively correlated are enriched with pathways involving translations, showing a quite different mechanism from previous studies. Moreover, the survival analysis reveals that higher VDR copy number aberration (CNA) or HER2 expression shows significantly higher death rates in breast cancer patients.

Conclusion: Our study reveals new mechanisms of coordinated function between VDR and HER2 in repressing cell translation, and prognosis or therapies targeted to VDR CNA and HER2 expression involving cell translations may be a potentially combined strategy in treating breast cancer.

Background

Breast cancer (BC), generally acknowledged as a heterogeneous and multifaceted disease, has a wide spectrum of clinical, pathologic, and molecular features [1–3]. Consequently, complete characterization and underlying technologies to predict its outcomes are remarkably generated on traditional clinicopathological features and tumor biomarkers such as human epidermal growth factor receptor 2 (HER2) [4]. HER2 protein overexpression or *HER2* gene amplification testing is a routine practice for newly diagnosed BC in pathology, as HER2-targeted therapies are beneficial to BC treatment. However, HER2-targeted therapies such as the addition of trastuzumab to chemotherapy still have scope for improvement in decelerating disease progression, increasing objective response rate, extending response duration and lowering death rate at 1 year. Interestingly, they also have a potentially severe adverse event of cardiotoxicity [5–13]. Owing to the heterogeneity of BC, other biological markers such as estrogen receptor (ER), progesterone receptor (PR), and Ki67 are indispensable to combine with HER2 in BC characterization [14, 15]. Approximately 15–25% of BC types consist of HER2-overexpression or *HER2* amplification, which promotes the growth of cancer cells [16]. HER2-targeted therapies are very effective for HER2-positive (HER2+) BC [7].

The steroid hormone, vitamin D, and its metabolic active form, $1,25(\text{OH})_2\text{D}_3$, play an important role in inhibiting the proliferation of BC cell lines and promoting their differentiation and apoptosis [17, 18].

Although vitamin D supplements have protective effects on BC [19], vitamin D intake by BC patients should refer to the expression level of their vitamin D receptor (VDR) [20]. VDR plays an important biological role in BC, specifically, triple-negative BC (TNBC), which is simply defined by the absence of ER and PR expression and the lack of *HER2* amplification [21–24]. In such cancers, validated targeted therapy options for ER, PR, and *HER2* are insensitive and have a poor prognosis with limited therapeutic options, and further improved therapies are required [25–28]. High incidences of this invasive BC subtype have been found in women of African descent because higher skin pigmentation is linked to lower sun absorption and reduced vitamin D synthesis [23, 29, 30]. Many studies have reported the effects and underlying mechanisms of VDR and its ligand, vitamin D, in TNBC, inferring many other factors. An ER-positive status and intact VDR signaling are both associated with vitamin D sensitivity in BC cells [31]; androgen receptor (AR) and VDR-targeted agonist hormone therapy can inhibit HR2-av TNBC through multiple mechanisms in a receptor-dependent manner and can be combined with chemotherapy [32]. However, the active forms of vitamin D, namely 25-hydroxyvitamin D3 (25-OH) and 1,25-dihydroxyvitamin D3 (1,25D3), have not shown any effects on three TNBC lines, MDA-MB-157, MDA-MB-231, and MDA-MB-468 [33]. Although studies regarding the anti-cancer effects of VDR and its ligand, 1,25D3, in TNBC have been reported, the underlying mechanisms are considerably heterogeneous [30, 32–40].

Since the roles of VDR and *HER2* in BC have been studied separately over the last few decades, research focusing on the relationship between VDR and *HER2* + BC and the functional mechanisms of their interaction is pivotal to study the role of VDR in BC therapies, which may lead to better overall survival. The specific relationship between VDR and *HER2* may provide a potential treatment for BC patients. This study aimed to determine: 1) the correlations between VDR and *HER2* and their underlying coordinated mechanisms in BC; and 2) the contributions of VDR and *HER2* to survival rates in BC, especially the expression and copy number aberration (CNA) of those two genes. By illustrating these, we hypothesize that factors that act on the interaction between VDR and *HER2* may be a potential target in BC therapies.

Results

Breast cancer patient characteristics related to *HER2* status from cBioPortal.

To analyze the association between VDR and *HER2* in BC, we obtained a total of 1904 BC patients with clinical prognosis characteristics, gene expression level, and CNA data from *cBioPortal* [41]. Measuring the association between expression/CNA of VDR/*HER2* and patients' characteristics can determine the combined effects of VDR and *HER2* in BC diagnosis and prognosis. The determined characteristics were divided into two groups: those related (Table 1) and unrelated (Table S1) to *HER2* status. In this dataset, 236 (12.39%) patients were *HER2*+, which is important for deciding between *HER2*-targeted therapies or drugs, and 417 (21.95%) patients gained *HER2* amplification status measured by SNP6 (Table 1). Other genes, such as claudins and hormone receptors, can also represent useful diagnostics and prognostics related to *HER2* status in BC [14, 42]. In this dataset, 220 (11.55%) patients were identified as *HER2* + in the claudin subtype, while 188 (11.06%) patients were classified into *HER2* + in a three-gene model (Table 1).

Table 1
Clinical characteristics related to HER2 status of breast cancer patients with mRNA expression data from *cBioPortal*.

Clinical characteristic (number)	Number	%
HER2 status (1904)		
Negative	1668	87.61
Positive	236	12.39
HER2 SNP6 (1900)		
Gain	417	21.95
Loss	100	5.26
Neutral	1383	72.79
Claudin subtype (1904)		
Basal	199	10.45
claudin-low	199	10.45
Her2	220	11.55
LumA	679	35.66
LumB	461	24.21
NC	6	0.32
Normal	140	7.35
Three-gene (1700)		
ER-/HER2-	290	17.06
ER+/HER2- High Prolif	603	35.47
ER+/HER2- Low Prolif	619	36.41
HER2+	188	11.06

As listed in Table S1, 15 clinical characteristics unrelated to HER2 status also depict the basic information of patients with BC progression. The median age of these patients was 61.77 years, ranging from 21.93 – 96.29 years. Among these cases, 9.01%, 40.30%, and 50.60% were in tumor Grade 1, 2, and 3, respectively, and most were in Stage 1 (33.86%) and Stage 2 (57.02%). The tumors have a size of 23, with a range of 1 – 182. Meanwhile, 47.85% (911/1904) of the patients in this study were diagnosed with positive lymph nodes. The Nottingham prognostic index (NPI), which is constructed by the tumor size, lymph node stage and pathological grade and is more discriminating than these characteristics alone

[43], ranges from 1 – 6.360, with a median value of 4.033. The ER/PR status determines the hormone receptor status, which is important for deciding treatment options. In this dataset, the ER status of the patients determined by two different methods are similar: 1459 (76.63%) by ER expression and 1445 (77.11%) by ER immunohistochemistry (IHC) were identified as ER-positive. Additionally, 1009 (52.99%) of the patients were identified as PR-positive.

VDR and HER2 show large co-effects on clinical prognosis characteristics

To infer the prognostic roles of VDR in correlation with HER2 in BC, we performed multivariate analysis between VDR expression/CNA and clinical prognosis characteristics, which are grouped with their relationship to HER2 status as listed in Table 1 and S1 (see Materials and Methods [44–46]). We also performed the same analysis between HER2 expression/CNA and the same characteristics as a control. Consequently, we detected a great association between HER2 expression or CNA and clinical characteristics of HER2 status, HER2 SNP6, Claudin subtype and three-gene (ANOVA test, all $P < 0.01$, Table 2). Simultaneously, VDR expression and CNA also show significant effects on clinical characteristics related to HER2 status as demonstrated by HER2 analysis (ANOVA tests, all $P < 0.01$, Table 2), except HER2 SNP6. For characteristics unrelated to HER2 status, expression and CNA of both VDR and HER2 show significant effects on ages and ER status (ANOVA tests, all $P < 0.05$, Table 3). These results imply that VDR and HER2 exert co-effects on BC. However, substantial differences still exist, that tumor grade, cellularity, and IntClust are significantly associated with VDR expression and tumor stage with VDR CNA; cellularity is significantly associated with HER2 expression and tumor size with HER2 CNA. To some extent, those differences provide evidence for the effect variation of VDR and HER2 on BC.

Table 2

Effects of VDR/HER2 expression or copy number alteration (CNA) on clinical prognosis characters.

Clinical prognosis		VDR		HER2	
Characters		Expression	CNA	Expression	CNA
Related to HER2 status	HER2 status	9.515 **	17.272 ***	3828.117 ***	2271.091 ***
	HER2 SNP6	1.368	1.342	90.463 ***	245.672 ***
	Claudin subtype	5.399 ***	6.297 ***	41.653 ***	3.034 **
	Three-gene	4.117 **	4.130 **	13.344 ***	5.773 ***
Unrelated to HER2 status	Age at diagnosis	4.423 *	7.817 **	41.615 ***	20.817 ***
	Grade	4.845 *	0.018	0.898	0.865
	Tumor size	0.705	1.143	0.295	7.925 **
	Tumor stage	2.416	3.978 *	0.585	0.043
	Lymph nodes examined positive	0.065	1.039	0.716	1.735
	NPI	0.907	3.357	0.460	2.578
	ER status	3.924 *	79.735 ***	44.590 ***	16.981 ***
	ER IHC	2.497	1.114	0.566	1.137
	PR status	2.424	0.227	30.125 ***	23.036 ***
	Cellularity	5.414 **	1.734	5.907 ***	0.476
	Cohort	0.133	0.006	73.919 ***	6.095 *
	Inferred menopausal state	1.024	0.220	0.379	0.255
	IntClust	2.799 **	1.804	7.566 ***	4.509 ***
	Histological subtype	0.845	1.371	0.618	0.340
	Laterality	0.669	0.830	0.685	0.021

Note: ANOVA *F*-values of single factor are shown, but that of interactions between/across factors are not shown. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3
Therapies and overall survival (OS) statistics of breast cancer patients.

Therapies/OS (number)	Number/median	%
Chemotherapy (1904)		
Yes	396	20.80
No	1508	79.20
Hormone therapy (1904)		
Yes	1174	61.66
No	730	38.34
Radiotherapy (1904)		
Yes	1137	40.28
No	767	59.72
Breast surgery (1882)		
Breast conserving	755	40.12
Mastectomy	1127	59.88
OS months (1904)		
Median OS months (range)	115.62 (0 ~ 355.20)	
OS status (1904)		
Living	801	42.07
Deceased	1103	57.93
Vital status (1903)		
Died of disease	622	32.69
Died of other causes	480	25.22
Living	801	42.09

A significantly positive correlation between VDR and HER2 status in breast tumors.

To further illustrate the association between VDR and HER2 in BC, we compared the VDR expression levels between HER2 status characterized by different clinicopathological features (Table 2). We observed that VDR was highly expressed in HER2 + cancers than HER2-negative ones (Mann-Whitney *U* test, $P < 0.001$, Fig. 1A), higher in the HER2 + tumors characterized in the three-gene model (Mann-Whitney *U* test, $P < 0.001$, Fig. 1B), higher in HER2 amplification gain characterized by SNP6 (Mann-Whitney *U* test,

$P < 0.01$, Fig. 1C), and higher in HER2-enriched cancer of the claudin subtype (Mann-Whitney U test, $P < 0.001$, Fig. 1D). These results suggest the positive association of VDR with HER2 in BC, which is verified by the correlation analysis between VDR and HER2 expression levels (Pearson's correlation, $r = 0.1686$, $P < 0.001$, Fig. 1E).

VDR and HER2 also appear to have similar significant effects on other clinical characteristics that have been widely exploited for targeted therapies and as signatures for outcomes in BC [47], such as age at diagnosis and ER status (Table 2). Although not all studies showed a significant correlation between VDR expression and age [48–51] in BC, we found a slightly negative correlation (Pearson's correlation $r = -0.06848$, $P = 0.002791$) (Figure S1A). Additionally, we observed a higher VDR expression in ER-negative than in ER-positive cancers (Mann – Whitney U test, $P < 0.01$, Figure S1B). Taken together, the positive correlations of VDR expression with clinical characteristics related to HER2 status, and the negative correlations unrelated to HER2 status, imply a coordinated function of VDR and HER2 in BC.

Genes negatively co-expressed with VDR and HER2 are enriched with pathways related to translations.

Next, we investigated the coordinated function of VDR and HER2 in BC. We identified genes that are positively or negatively correlated with HER2 or VDR expression using a linear regression model in the whole transcriptome (see *Materials and Methods*). In this dataset, we identified 5105 and 5540 genes positively and negatively correlated with HER2 expression level, respectively, and 3762 and 3599 genes positively and negatively correlated with VDR expression, respectively (Figure. 2A). A total of 58.9% (2217/3762) of genes were positively correlated with both HER2 and VDR expressions, and 66.6% (2397/3599) were negatively correlated (Figure. 2B). To illustrate the underlying mechanisms of VDR and HER2 coordination, we performed functional enrichment analysis on the overlapping genes using ReactomeFIViz in Cytoscape (v3.8.2) [52]. Surprisingly, the overlapping negatively related genes were found to be mainly enriched in pathways related to translation, such as “GTP hydrolysis and joining of the 60S ribosomal subunit,” “Eukaryotic Translation Termination,” “Eukaryotic Translation Initiation,” “Eukaryotic Translation Elongation,” “Selenocysteine synthesis,” “L13a-mediated translational silencing of Ceruloplasmin expression,” “Peptide chain elongation,” “Formation of a pool of free 40S subunits,” and “Cap-dependent Translation Initiation.” Pathways related to nonsense-mediated decay were also enriched, such as “Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)” and “Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC).” Only three pathways were enriched in the overlapping positively related genes: “Asparagine N – linked glycosylation,” “ER to Golgi Anterograde Transport,” and “Transport to the Golgi and subsequent modification.” Our results uncover the underlying mechanism that VDR and HER2 function coordinately to mainly repress translation in BC.

Lower VDR CNA shows better survival in breast cancer.

The prognostic importance of VDR and HER2 can be measured by the survival analysis and Cox proportional hazards models. In the dataset used, 396, 1174, 1137, and 755 patients received chemotherapy, hormone therapy, radiotherapy, and breast-conserving surgery, respectively (Table 3). The

median overall survival (OS months) was 115.62 months (range, 0–355.20, Table 2). Of the 1103 (57.93%) deceased patients, 622 died of BC (OS and Vital status, Table 2). Then, we applied the survival analysis of VDR/HER2 expression/CNA in BC, taking the aforementioned therapies into consideration (see *Materials and Methods*). As shown in Table 4, chemotherapy, hormone therapy, VDR CNA, and HER2 expression were associated with a higher risk of death (positive coefficients and $P < 0.05$), but breast-conserving surgery was associated with a lower risk of death (negative coefficients and $P < 0.05$). Moreover, elevated HER2 expression and VDR CNA showed lower survival rates in BC. In contrast to the relative similarity of survival curves between high and low HER2 expression levels, VDR showed larger differences across different CNAs. Our results reveal that the combined effect of VDR CNA and HER2 expression may be a good target for BC therapies or drugs.

Table 4
Survival analysis based on VDR and HER2 in breast cancer.

Variables	coef	z	Pr(> z)
Chemotherapy	0.2152	2.596	0.009431 **
Hormone therapy	0.2456	3.752	0.0001760 ***
Radiotherapy	-0.007386	-0.097	0.9229
Breast conserving	-0.8290	-2.842	0.004485 **
Breast mastectomy	-0.3900	-1.352	0.1763
VDR expression	-0.09806	-1.010	0.3125
VDR CNA	0.1650	2.066	0.03885 *
HER2 expression	0.07617	2.071	0.03836 *
HER2 CNA	-0.006529	-0.120	0.9045
Note: Cox Proportional Hazards regression were performed. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.			

Discussion

Low vitamin D levels are one of the factors that lead to the poor diagnosis of BC, especially in women of African descent who have a higher incidence of cancer with more aggressive features [29]. These aggressive features include negative ER, PR, and HER2 expression, which reflects TNBC that is unresponsive to targeted therapies or drugs and leads to poor prognosis. The therapeutic effects of vitamin D levels in TNBC have been widely studied [29, 32, 33]. The relationship between VDR expression and TNBC and its role in prognosis has also been studied [53, 54]. Despite VDR expression levels, VDR polymorphisms were also associated with the molecular mechanism of BC susceptibility [55–63]. These studies were mostly limited to a few SNPs or cases. Based on large-scale BC data, our study shows that VDR expression and CNA are associated with triple-negative cancers. A negative association with ER

status and positive association with HER2 status revealed coordinated functions between VDR and HER2, on which studies are largely limited.

Although studies on translation regulation and NMD in BC have been reported [64–72], further research on the mechanisms of VDR and HER2 focusing on pathways in TNBC is still required. MiRNAs mediated post-transcriptional regulation of VDR or HER2 in BC present another research avenue regarding the coordinated mechanisms [73–76], especially those regulating VDR and HER2 simultaneously. MiR-125b is a miRNA co-targeted to both VDR and HER2 (TargetScan, release 7.2 [77]) and reported as a tumor repressor in BC [78–80]. In addition, many other factors also act on translation in BC. BRCA1 (BRCA1 (Breast Cancer gene 1)) acts as a tumor repressor and plays a key role in breast and ovarian cancer pathogenesis [81, 82]. In *in vitro* transcription/translation assays, translation efficiency was 30 – 50% lower in the mutated *BRCA1* 5'UTR than in the wild-type *BRCA1* 5'UTR [83]. Our studies provide theoretical evidence to those studies and point to an intriguing direction in mechanistic studies of TNBCs.

Finally, most studies involving VDR and the BC progression and OS have evaluated VDR expression [53, 84–86]. In our survival analysis, we used VDR CNA instead of its expression, which affects survival rates (Table 4 and Fig. 4). Although VDR expression was highly correlated with VDR CNA (ANOVA, $F = 8.11$, $P = 0.00445$, Figure S1), future studies or therapies may point to VDR CNA. As a member of the epidermal growth factor family, HER2 is an upstream component of the pi3k-Akt pathway. Amplification of this receptor has been shown to play a vital role in the growth and progression of certain aggressive BC types [87, 88]. In fact, about 20% (1 in 5) of BC patients are found to have HER2 overexpression, which is related to a very poor prognosis [16]. This specific overexpression also affects survival [13, 89–92]; this was also verified in our study (Table 4 and Fig. 4).

Conclusion

Our study shows a significant positive specific correlation between VDR and HER2, indicating a potential new treatment for HER2 + patients. Moreover, a relatively high proportion of overlapping genes that are significantly correlated with VDR and HER2 expression levels was detected in BC (58.9%, positive; 66.6%, negative). Genes negatively correlated with both VDR and HER2 expressions are functionally enriched with pathways involving cell translations, providing a direction for the further development of new therapeutic strategies. Notably, the HER2 expression and VDR CNA show a significant difference in the survival rate of BC. Our studies provide theoretical evidence to combined therapies focused on HER2 expression and VDR CNA in BC.

Materials And Methods

Breast cancer dataset

BC datasets were obtained from *cBioPortal* [41, 44–46]. The datasets included mRNA expression (data_expression_median.txt, number of samples $n = 1904$), information on clinical samples

(data_clinical_sample.txt, n = 2509; data_clinical_patients.txt, n = 2509), and copy number aberration (CNA) (data_CNA.txt, n = 2173). These data were combined according to the clinical samples (16,823 genes and 1904 samples) and used for subsequent analyses.

Association Analysis Between Vdr/her2 And Clinical Prognosis Characteristics

Multivariate analysis of variance (ANOVA) was used to evaluate associations between VDR/HER2 expression/CNA and clinical prognosis characteristics, including ER, HER2, and PR statuses; tumor size, stage, and grade; positive lymph nodes; Nottingham prognostic index (NPI, which is calculated using the tumor size, number of involved lymph nodes, and tumor grade); cellularity (proportion of tumor volume occupied by invasive tumor cells); family cohort; ER immunohistochemistry (IHC); HER2 SNP6; claudin subtype; inferred menopausal state; three-gene model; IntClust; histological subtype; and laterality. Data on VDR and HER2 expression levels in clinical samples were extracted from data_expression_median.txt, and CNAs were extracted from data_CNA.txt, while clinical prognosis characteristics were obtained from data_clinical_sample.txt and data_clinical_patients.txt. Given the expression level/CNA is the variable y and the i th clinical variable is x_i , ANOVA was performed as follows using the R program (<https://www.r-project.org/>): $\text{fit} = \text{aov}(y \sim x_1 + x_2 + \dots + x_i)$.

Association analysis between VDR and HER2

First, we used ANOVA to compare the expression level and CNA of VDR and HER2 using the “ $\text{fit} = \text{aov}(\text{expression} \sim \text{CNA})$ ” function to confirm the association between expression level and CNA. Thereafter, linear regression of expression level between VDR and HER2 was performed with “ $\text{fit} = \text{lm}(\text{VDR} \sim \text{HER2})$ ” function using the R program [93].

Whole-transcriptome association analysis with VDR/HER2 expression and functional enrichment analysis for pathways

Linear regression analysis and Benjamini–Hochberg multiple testing correction (FDR) were used to test the correlation between the expression of each gene in the transcriptome and that of VDR and HER2, respectively. Genes with $\text{FDR} < 0.05$ and Pearson’s $r > 0$ were considered positively associated with VDR and HER2, while those with $\text{FDR} < 0.05$ and Pearson’s $r < 0$ were considered negatively associated. Genes negatively or positively associated with both VDR and HER2 were subsequently used to perform functional enrichment using ReactomeFIViz in Cytoscape (v 3.8.2, [52]). Pathways with $\text{FDR} < 0.05$ were identified as enriched.

Survival Analysis

The Cox Proportional-Hazards Model was used to perform survival analysis in BC as “fit <- coxph(Surv(OS months, OS status) ~ chemotherapy + hormone therapy + radiotherapy + breast surgery + VDR expression + VDR CNA + HER2 expression + HER2 CNA)” using the R packages “survival” and “survminer.” We determined OS months as the survival time and OS status as the survival status, and clinical characteristics of the patients, including chemotherapy, hormone therapy, radiotherapy, and breast surgery, were taken into consideration. Survival curves were plotted using the R functions “survfit” and “Surv,” and HER2 expressions were categorized as “low” and “high” according to the median value [93].

Abbreviations

BC

breast cancer

VDR

vitamin D receptor

HER2

human epidermal growth factor receptor 2

CNA

copy number aberration

ER

estrogen receptor

PR

progesterone receptor

TNBC

triple-negative breast cancer

NMD

nonsense-mediated decay

EJC

exon junction complex

OS

overall survival

NPI

Nottingham prognostic index

ER IHC

ER immunohistochemistry

ANOVA

analysis of variance

BRCA1

BRest CAncer gene 1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the *cBioPortal* repository (https://www.cbioportal.org/study/summary?id=brca_metabric) [41, 44-46].

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no external funding.

Authors' contributions

HR, CD and AD designed the research study. HR performed the research and analyzed the data. HR and AD wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank Xu He for suggestions on project conceptualization and academic writing tutoring. We also would like to thank Editage (www.editage.cn) for English language editing.

References

1. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490:61–70.
2. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler 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.
3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA*. 2006;295:2492–502.
4. Cadoo KA, Traina TA, King TA. Advances in molecular and clinical subtyping of breast cancer and their implications for therapy. *Surg Oncol Clin N Am*. 2013;22:823–40.

5. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001;344:783–92.
6. Watanabe S, Yonesaka K, Tanizaki J, Nonagase Y, Takegawa N, Haratani K, et al. Targeting of the HER2/HER3 signaling axis overcomes ligand-mediated resistance to trastuzumab in HER2-positive breast cancer. *Cancer Med*. 2019;8:1258–68.
7. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of Women With Metastatic Breast Cancer by HER2 Status and Trastuzumab Treatment: An Institutional-Based Review. *J Clin Oncol*. 2010;28:92–8.
8. Tovey SM, Brown S, Doughty JC, Mallon EA, Cooke TG, Edwards J. Poor survival outcomes in HER2-positive breast cancer patients with low-grade, node-negative tumours. *Br J Cancer*. 2009;100:680–3.
9. Sendur MA, Aksoy S, Altundag K. Pertuzumab in HER2-positive breast cancer. *Curr Med Res Opin*. 2012;28:1709–16.
10. Wuerstlein R, Harbeck N. Neoadjuvant Therapy for HER2-positive Breast Cancer. *Reviews on Recent Clinical Trials*. 2017;12:81–92.
11. Sun X, Wang X-E, Zhang Z-P, Shi Z-Q, Cong B-B, Wang Y-S, et al. Neoadjuvant therapy and sentinel lymph node biopsy in HER2-positive breast cancer patients: results from the PEONY trial. *Breast Cancer Res Treat*. 2020;180:423–8.
12. Gingras I, Gebhart G, de Azambuja E, Piccart-Gebhart M. HER2-positive breast cancer is lost in translation: time for patient-centered research. *Nature Reviews Clinical Oncology*. 2017;14:669–81.
13. López-Guerrero JA, Llombart-Cussac A, Noguera R, Navarro S, Pellin A, Almenar S, et al. HER2 amplification in recurrent breast cancer following breast-conserving therapy correlates with distant metastasis and poor survival. *Int J Cancer*. 2006;118:1743–9.
14. Parise CA, Caggiano V. Breast Cancer Survival Defined by the ER/PR/HER2 Subtypes and a Surrogate Classification according to Tumor Grade and Immunohistochemical Biomarkers. *J Cancer Epidemiol*. 2014; 2014:469251.
15. Meric-Bernstam F, Johnson AM, Dumbrava EEI, Raghav K, Balaji K, Bhatt M, et al. Advances in HER2-Targeted Therapy: Novel Agents and Opportunities Beyond Breast and Gastric Cancer. *Clin Cancer Res*. 2019;25:2033–41.
16. Slamon D, Clark G, Wong S, Levin W, Ullrich A, McGuire W. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177–82.
17. So JY, Lee HJ, Smolarek AK, Paul S, Wang CX, Maehr H, et al. A novel Gemini vitamin D analog represses the expression of a stem cell marker CD44 in breast cancer. *Mol Pharmacol*. 2011;79:360–7.
18. Verma A, Cohen DJ, Schwartz N, Muktipaty C, Koblinski JE, Boyan BD, et al. 24R,25-Dihydroxyvitamin D3 regulates breast cancer cells in vitro and in vivo. *Biochim Biophys Acta Gen Subj*. 2019;1863:1498–512.

19. Madden JM, Murphy L, Zgaga L, Bennett K. De novo vitamin D supplement use post-diagnosis is associated with breast cancer survival. *Breast Cancer Res Treat.* 2018;172:179–90.
20. Xu H, Liu Z, Shi H, Wang C. Prognostic role of vitamin D receptor in breast cancer: a systematic review and meta-analysis. *BMC Cancer.* 2020;20:1051.
21. Lehmann BD, Pietersen JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. *J Pathol.* 2014;232:142–50.
22. Abramson VG, Lehmann BD, Ballinger TJ, Pietersen JA. Subtyping of triple-negative breast cancer: implications for therapy. *Cancer.* 2015;121:8–16.
23. Kumar P, Aggarwal R. An overview of triple-negative breast cancer. *Arch Gynecol Obstet.* 2016;293:247–69.
24. Sporikova Z, Koudelakova V, Trojanec R, Hajduch M. Genetic Markers in Triple-Negative Breast Cancer. *Clin Breast Cancer.* 2018;18:e841–50.
25. Lyons TG. Targeted Therapies for Triple-Negative Breast Cancer. *Curr Treat Options Oncol.* 2019;20:82.
26. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res.* 2020;22:61.
27. Navratil J, Fabian P, Palacova M, Petrakova K, Vyzula R, Svoboda M. [Triple Negative Breast Cancer]. *Klin Onkol.* 2015;28:405–15.
28. Medina MA, Oza G, Sharma A, Arriaga LG, Hernandez Hernandez JM, Rotello VM, et al. Triple-Negative Breast Cancer: A Review of Conventional and Advanced Therapeutic Strategies. *Int J Environ Res Public Health.* 2020; 17.
29. Yao S, Ambrosone CB. Associations between vitamin D deficiency and risk of aggressive breast cancer in African-American women. *J Steroid Biochem Mol Biol.* 2013;136:337–41.
30. Qin B, Xu B, Ji N, Yao S, Pawlish K, Llanos AAM, et al. Intake of vitamin D and calcium, sun exposure, and risk of breast cancer subtypes among black women. *Am J Clin Nutr.* 2020;111:396–405.
31. Peng X, Jhaveri P, Hussain-Hakimjee EA, Mehta RG. Overexpression of ER and VDR is not sufficient to make ER-negative MDA-MB231 breast cancer cells responsive to 1alpha-hydroxyvitamin D5. *Carcinogenesis.* 2007; 28:1000–1007.
32. Thakkar A, Wang B, Picon-Ruiz M, Buchwald P, Ince TA. Vitamin D and androgen receptor-targeted therapy for triple-negative breast cancer. *Breast Cancer Res Treat.* 2016;157:77–90.
33. Richards SE, Weierstahl KA, Kelts JL. Vitamin D effect on growth and vitamin D metabolizing enzymes in triple-negative breast cancer. *Anticancer Res.* 2015;35:805–10.
34. Blasiak J, Pawlowska E, Chojnacki J, Szczepanska J, Fila M, Chojnacki C. Vitamin D in Triple-Negative and BRCA1-Deficient Breast Cancer-Implications for Pathogenesis and Therapy. *Int J Mol Sci.* 2020; 21.
35. Narvaez CJ, Grebenc D, Balint S, Welsh JE. Vitamin D regulation of HAS2, hyaluronan synthesis and metabolism in triple negative breast cancer cells. *J Steroid Biochem Mol Biol.* 2020;201:105688.

36. Wilhelm CA, Clor ZJ, Kelts JL. Effect of Vitamin D on Paclitaxel Efficacy in Triple-negative Breast Cancer Cell Lines. *Anticancer Res.* 2018;38:5043–8.
37. Abdel-Mohsen MA, Abo Deif SM, Abou-Shamaa LA. IL-6 Impairs the Activity of Vitamin D3 in the Regulation of Epithelial-Mesenchymal Transition in Triple Negative Breast Cancer. *Asian Pac J Cancer Prev.* 2019;20:2267–73.
38. LaPorta E, Welsh J. Modeling vitamin D actions in triple negative/basal-like breast cancer. *J Steroid Biochem Mol Biol.* 2014; 144 Pt A:65–73.
39. Martinez-Reza I, Diaz L, Barrera D, Segovia-Mendoza M, Pedraza-Sanchez S, Soca-Chafre G, et al. Calcitriol Inhibits the Proliferation of Triple-Negative Breast Cancer Cells through a Mechanism Involving the Proinflammatory Cytokines IL-1beta and TNF-alpha. *J Immunol Res.* 2019; 2019:6384278.
40. Soljic M, Mrklic I, Tomic S, Omrcen T, Sutalo N, Bevanda M, et al. Prognostic value of vitamin D receptor and insulin-like growth factor receptor 1 expression in triple-negative breast cancer. *J Clin Pathol.* 2018;71:34–9.
41. The *cBioPortal*. https://www.cbioportal.org/study/summary?id=brca_metabric. Accessed 20 August 2021.
42. Lu S, Singh K, Mangray S, Tavares R, Noble L, Resnick MB, et al. Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype. *Mod Pathol.* 2013;26:485–95.
43. Haybittle JL, Blamey RW, Elston CW, Johnson J, Doyle PJ, Campbell FC, et al. A prognostic index in primary breast cancer. *Br J Cancer.* 1982;45:361–6.
44. Pereira B, Chin SF, Rueda OM, Vollan HK, Provenzano E, Bardwell HA, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat Commun.* 2016;7:11479.
45. Rueda OM, Sammut S-J, Seoane JA, Chin S-F, Caswell-Jin JL, Callari M, et al. Dynamics of breast-cancer relapse reveal late-recurring ER-positive genomic subgroups. *Nature.* 2019;567:399–404.
46. Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature.* 2012;486:346–52.
47. Early Breast Cancer Trialists'. Collaborative G, Davies C, Godwin J, Gray R, Clarke M, Cutter D, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet.* 2011; 378:771–84.
48. Jiang Y, Liao L, Li J, Wang L, Xie Z. Older Age Is Associated with Decreased Levels of VDR, CYP27B1, and CYP24A1 and Increased Levels of PTH in Human Parathyroid Glands. *Int J Endocrinol.* 2020; 2020:7257913.
49. Tong T, Liu Z, Zhang H, Sun J, Zhang D, Wang F, et al. Age-dependent expression of the vitamin D receptor and the protective effect of vitamin D receptor activation on H2O2-induced apoptosis in rat intervertebral disc cells. *J Steroid Biochem Mol Biol.* 2019;190:126–38.

50. Coleman LA, Mishina M, Thompson M, Spencer SM, Reber AJ, Davis WG, et al. Age, serum 25-hydroxyvitamin D and vitamin D receptor (VDR) expression and function in peripheral blood mononuclear cells. *Oncotarget*. 2016;7:35512–21.
51. Yao X, Ei-Samahy MA, Yang H, Feng X, Li F, Meng F, et al. Age-associated expression of vitamin D receptor and vitamin D-metabolizing enzymes in the male reproductive tract and sperm of Hu sheep. *Anim Reprod Sci*. 2018;190:27–38.
52. Shannon P. Cytoscape. A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res*. 2003;13:2498–504.
53. Huss L, Butt ST, Borgquist S, Elebro K, Sandsveden M, Rosendahl A, et al. Vitamin D receptor expression in invasive breast tumors and breast cancer survival. *Breast Cancer Res*. 2019;21:84.
54. Al-Azhri J, Zhang Y, Bshara W, Zirpoli G, McCann SE, Khoury T, et al. Tumor Expression of Vitamin D Receptor and Breast Cancer Histopathological Characteristics and Prognosis. *Clin Cancer Res*. 2017;23:97–103.
55. Raza S, Dhasmana A, Bhatt MLB, Lohani M, Arif JM. Molecular Mechanism of Cancer Susceptibility Associated with Fok1 Single Nucleotide Polymorphism of VDR in Relation to Breast Cancer. *Asian Pac J Cancer Prev*. 2019;20:199–206.
56. Ahmed JH, Makonnen E, Fotoohi A, Yimer G, Seifu D, Assefa M, et al. Vitamin D Status and Association of VDR Genetic Polymorphism to Risk of Breast Cancer in Ethiopia. *Nutrients*. 2019; 11.
57. Shaikh F, Baig S, Jamal Q. Do VDR Gene Polymorphisms Contribute to Breast Cancer? *Asian Pac J Cancer Prev*. 2016;17:479–83.
58. Mohseni H, Amani R, Hosseini SA, Ekrami A, Ahmadzadeh A, Latifi SM. Genetic Variations in VDR could Modulate the Efficacy of Vitamin D3 Supplementation on Inflammatory Markers and Total Antioxidant Capacity among Breast Cancer Women: A Randomized Double Blind Controlled Trial. *Asian Pac J Cancer Prev*. 2019;20:2065–72.
59. Matini AH, Jafarian-Dehkordi N, Bahmani B, Sharifi M, Jahantigh D, Mazoochi T. Association of ApaI and TaqI polymorphisms in VDR Gene with Breast Cancer. *Asian Pac J Cancer Prev*. 2020;21:2667–72.
60. Lu D, Jing L, Zhang S. Vitamin D Receptor Polymorphism and Breast Cancer Risk: A Meta-Analysis. *Medicine*. 2016;95:e3535.
61. Rai V, Abdo J, Agrawal S, Agrawal DK. Vitamin D Receptor Polymorphism and Cancer: An Update. *Anticancer Res*. 2017;37:3991–4003.
62. Francis I, AlAbdali N, Kapila K, John B, Al-Temaimi RA. Vitamin D pathway related polymorphisms and vitamin D receptor expression in breast cancer. *Int J Vitam Nutr Res*. 2021;91:124–32.
63. Shahabi A, Alipour M, Safiri H, Tavakol P, Alizadeh M, Milad Hashemi S, et al. Vitamin D Receptor Gene Polymorphism: Association with Susceptibility to Early-Onset Breast Cancer in Iranian, BRCA1/2-Mutation Carrier and non-carrier Patients. *Pathol Oncol Res*. 2018;24:601–7.
64. Ochnik AM, Peterson MS, Avdulov SV, Oh AS, Bitterman PB, Yee D. Amplified in Breast Cancer Regulates Transcription and Translation in Breast Cancer Cells. *Neoplasia*. 2016;18:100–10.

65. Gomez-Cambronero J. Lack of effective translational regulation of PLD expression and exosome biogenesis in triple-negative breast cancer cells. *Cancer Metastasis Rev.* 2018;37:491–507.
66. Jewer M, Lee L, Leibovitch M, Zhang G, Liu J, Findlay SD, et al. Translational control of breast cancer plasticity. *Nat Commun.* 2020;11:2498.
67. Jones RA, Robinson TJ, Liu JC, Shrestha M, Voisin V, Ju Y, et al. RB1 deficiency in triple-negative breast cancer induces mitochondrial protein translation. *J Clin Invest.* 2016;126:3739–57.
68. Dacheux E, Vincent A, Nazaret N, Combet C, Wierinckx A, Mazoyer S, et al. BRCA1-Dependent Translational Regulation in Breast Cancer Cells. *PLoS One.* 2013;8:e67313.
69. Meric-Bernstam F, Chen H, Akcakanat A, Do KA, Lluch A, Hennessy BT, et al. Aberrations in translational regulation are associated with poor prognosis in hormone receptor-positive breast cancer. *Breast Cancer Res.* 2012;14:R138.
70. Bronson MW, Hillenmeyer S, Park RW, Brodsky AS. Estrogen coordinates translation and transcription, revealing a role for NRSF in human breast cancer cells. *Mol Endocrinol.* 2010;24:1120–35.
71. Li S, Ma J, Hu C, Zhang X, Xiao D, Hao L, et al. The Novel Pathogenic Mutation c.849dupT in BRCA2 Contributes to the Nonsense-Mediated mRNA Decay of BRCA2 in Familial Breast Cancer. *J Breast Cancer.* 2018;21:330–3.
72. Johnson JK, Waddell N, kConFab I, Chenevix-Trench G. The application of nonsense-mediated mRNA decay inhibition to the identification of breast cancer susceptibility genes. *BMC Cancer.* 2012;12:246.
73. Liu X, Bi L, Wang Q, Wen M, Li C, Ren Y, et al. miR-1204 targets VDR to promotes epithelial-mesenchymal transition and metastasis in breast cancer. *Oncogene.* 2018;37:3426–39.
74. Xiao Y, Humphries B, Yang C, Wang Z. MiR-205 Dysregulations in Breast Cancer: The Complexity and Opportunities. *Noncoding RNA.* 2019; 5.
75. Singh T, Adams BD. The regulatory role of miRNAs on VDR in breast cancer. *Transcription.* 2017;8:232–41.
76. McGuire A, Brown JA, Kerin MJ. Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. *Cancer Metastasis Rev.* 2015;34:145–55.
77. TargetScan. http://www.targetscan.org/vert_72/. Accessed Sep 23 2021.
78. Wang Y, Zeng G, Jiang Y. The Emerging Roles of miR-125b in Cancers. *Cancer Manag Res.* 2020;12:1079–88.
79. Hu G, Zhao X, Wang J, Lv L, Wang C, Feng L, et al. miR-125b regulates the drug-resistance of breast cancer cells to doxorubicin by targeting HAX-1. *Oncol Lett.* 2018;15:1621–9.
80. Feliciano A, Castellvi J, Artero-Castro A, Leal JA, Romagosa C, Hernandez-Losa J, et al. miR-125b acts as a tumor suppressor in breast tumorigenesis via its novel direct targets ENPEP, CK2-alpha, CCNJ, and MEGF9. *PLoS One.* 2013;8:e76247.
81. Duffy MJ, Walsh S, McDermott EW, Crown J. Biomarkers in Breast Cancer. 2015;71:1–23.

82. Romagnolo A, Romagnolo D, Selmin O. BRCA1 as Target for Breast Cancer Prevention and Therapy. *Anticancer Agents Med Chem.* 2014;15:4–14.
83. Signori E, Bagni C, Papa S, Primerano B, Rinaldi M, Amaldi F, et al. A somatic mutation in the 5'UTR of BRCA1 gene in sporadic breast cancer causes down-modulation of translation efficiency. *Oncogene.* 2001;20:4596–600.
84. Zati Zehni A, Jacob SN, Mumm JN, Heidegger HH, Ditsch N, Mahner S, et al. Hormone Receptor Expression in Multicentric/Multifocal versus Unifocal Breast Cancer: Especially the VDR Determines the Outcome Related to Focality. *Int J Mol Sci.* 2019; 20.
85. Ditsch N, Toth B, Mayr D, Lenhard M, Gallwas J, Weissenbacher T, et al. The association between vitamin D receptor expression and prolonged overall survival in breast cancer. *J Histochem Cytochem.* 2012;60:121–9.
86. Murray A, Madden SF, Synnott NC, Klinger R, O'Connor D, O'Donovan N, et al. Vitamin D receptor as a target for breast cancer therapy. *Endocr Relat Cancer.* 2017;24:181–95.
87. Ruiz-Saenz A, Dreyer C, Campbell MR, Steri V, Gulizia N, Moasser MM. HER2 Amplification in Tumors Activates PI3K/Akt Signaling Independent of HER3. *Cancer Res.* 2018;78:3645–58.
88. Rohlenova K, Neuzil J, Rohlena J. The role of Her2 and other oncogenes of the PI3K/AKT pathway in mitochondria. *Biol Chem.* 2016;397:607–15.
89. Borg A, Tandon AK, Sigurdsson H, Clark GM, Fernö M, Fuqua SA, et al. HER-2/neu amplification predicts poor survival in node-positive breast cancer. *Cancer Res.* 1990;50:4332–7.
90. Seshadri R, Figgairi FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. *J Clin Oncol.* 1993;11:1936–42.
91. Cho EY, Choi YL, Han JJ, Kim KM, Oh YL. Expression and amplification of Her2, EGFR and cyclin D1 in breast cancer: immunohistochemistry and chromogenic in situ hybridization. *Pathol Int.* 2008;58:17–25.
92. Pauley RJ, Gimotty PA, Paine TJ, Dawson PJ, Wolman SR. INT2 and ERBB2 amplification and ERBB2 expression in breast tumors from patients with different outcomes. *Breast Cancer Res Treat.* 1996;37:65–76.
93. The R Project for Statistical Computing. <https://www.r-project.org/>. Accessed August 20 2021.

Figures

Figure 1

Association studies between VDR and HER2 status in breast cancers. (A) Comparison of VDR expression level between HER2-negative and HER2-positive (HER2+) cancers. (B) Comparison of VDR expression level between HER2+ cells and other types of three-gene model. (C) Comparison of VDR expression level among HER2 amplification measured by SNP6. (D) Comparison of VDR expression level between HER2-enriched (Her2) cells and other types of Claudin subtype. Mann-Whitney U tests were performed, ** $P <$

0.01; *** $P < 0.001$. (E) Linear regression between HER2 and VDR expressions. Pearson's correlation (r) and P-value are shown in-frame.

Figure 2

Overlapping effects of HER2 and VDR expressions. (A) Volcano plots of linear regression analysis between HER2/VDR expression and other genes. Genes with positive correlations are indicated in red, those with negative correlations are indicated in blue, while non-significant genes are indicated in grey. (B) Venn diagram of VDR/HER2 positive and negative genes.

Figure 3

Functional enrichments of Reactome Pathway. Enriched pathways of positive genes are indicated in red, while those of negative genes are indicated in blue.

Figure 4

Survival curves of HER2 expression (A) and VDR CNA (B).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Sl.docx](#)