RESEARCH ARTICLE

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Bioinformatics Analysis of Programmed Death-1–Trastuzumab Resistance Regulatory Networks in Breast Cancer Cells

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Abstract

Objective: Programmed cell death-1 (PD-1, encoded by *PDCD1*) regulatory network participates in glioblastoma multiforme development. However, such a network in trastuzumab-resistant human epidermal growth factor receptor 2-positive (HER2+) breast cancer remains to be determined. Accordingly, this study was aimed to explore the PD-1 regulatory network responsible for the resistance of breast cancer cells to trastuzumab through a bioinformatics approach. **Methods:** The study used data mining tools like cBioportal and OMIM to identify genes involved in the programmed cell death-1–trastuzumab resistance regulatory network. The network was further examined using various tools like WebGestalt, DAVID, STRING, Cytoscape, CytoHubba, GEPIA, TNMPlot, and ROCPlot**. Results:** The *PDCD1* regulatory network in trastuzumab-resistant HER2+ breast cancer is linked to Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), interleukin (IL)-10, protein tyrosine phosphatase receptor type C (PTPRC), and FCGR2B. These factors have a significant prognostic power in pathological complete response in breast cancer patients treated with trastuzumab. Infiltration of B cells, CD8+ cells, CD4+ cells, neutrophils, dendritic cells, macrophages, and regulatory T cells is directly correlated with PTR expression. **Conclusion:** The study identified four genes (*CTLA4, IL10, PTPRC,* and *FCGR2B*) that are linked to the regulatory network of PD-1 in trastuzumab-resistant HER2+ breast cancer cells. Further research is needed to develop the therapeutic target against trastuzumab resistance in HER2+ breast cancer.

Keywords: PD-1- breast cancer- trastuzumab resistance- HER2+- bioinformatics- targeted therapy

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Introduction

Trastuzumab is the first-choice for HER2+ breast cancer therapy [1], and accounts for 30% of all breast cancer cases [2]. However, the remission rates are approximately 30%, and patients experience relapse within a year [3]. Therefore, a combinatorial therapeutic strategy must be developed to assess the effectiveness of trastuzumab. Breast cancer cells exhibit resistance to trastuzumab through various mechanisms, including deactivation of the Phosphatase and tensin homolog (PTEN) pathway, emergence of compensatory pathways, and downstream activation of HER2 signaling in the absence of ligands due to crosstalk with other receptor signaling pathways, such as the estrogen receptor (ER), and epigenetic mechanisms regulated by miRNAs [4-7]. Trastuzumab resistance is also due to the failure to stimulate immune-mediated cytotoxicity [8]. With the continuous identification of novel cancer hallmarks, novel mechanisms of drug discovery and development are also warranted to overcome trastuzumab resistance.

Programmed cell death-1 (PD-1), encoded by programmed cell death-1 (*PDCD1*), is an immunoregulatory molecule used as a therapeutic target in cancer [9]. PD-1 serves as an oncogene that protects T cells from killing other cells [10]. The PD-1 regulatory network plays a role in glioblastoma multiforme [11]. A previous study performed genomic and immunohistochemical analyses to predict the biomarkers of trastuzumab-elicited tumor immune response and found that the expression of PD-1-associated genes (including *CD274, IGHG1*, *CXCL10, IDO1, S100A9, CXCL9, CYP4Z2P, KIT, SALL4, MATN2,* and *CACNA1G*) in neoadjuvant trastuzumab-treated tumors correlates with the survival of patients with HER2+ breast cancer [12]. However, the regulatory network of PD-1 in trastuzumab-resistant HER2+ breast cancer is unknown.

In this study, we employed a bioinformatics approach to explore the PD-1 regulatory network responsible for the resistance of breast cancer cells to trastuzumab. The study implemented data mining tools such as OMIM and cBioportal to identify genes that are implicated in

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the programmed cell death-1–trastuzumab resistance regulatory network. The network was further investigated using a variety of tools, including WebGestalt, DAVID, STRING, Cytoscape, CytoHubba, GEPIA, TNMPlot, and ROCPlot. Our results indicate the potential role of PD-1 in the trastuzumab resistance in breast cancer. Moreover, we discussed the potential of regulatory networks related to PD-1 in overcoming breast cancer resistance to trastuzumab.

Materials and Methods

Data mining

Coexpressed genes against *PDCD1* were downloaded from the TCGA dataset from cBioportal (https://www.cbioportal.org/) [13], the keywords "*PDCD1*" and "breast cancer." Genes with Spearman correlation coefficient values >0.4 or −0.4 were selected. Regulatory genes of trastuzumab resistance in breast cancer were obtained from OMIM (https://www.omim.org/) [14] with the keyword "trastuzumab resistance." The intersection of data from cBioportal and OMIM was retrieved using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) [15], and data were considered genes involved in the *PDCD1*–trastuzumab resistance regulatory network (PTR).

Gene ontology and drug–gene association analysis

Gene ontology (GO) enrichment and drug–gene association analyses of PTR were conducted using over representative analysis (ORA) of WebGestalt (http://www.webgestalt.org/process.php) [16]. PTR was submitted as a query for ORA, and $P < 0.05$ was used as a selection criterion.

KEGG pathway enrichment analysis

PTR was analyzed for KEGG pathway enrichment using DAVID version 6.8 (https://david.ncifcrf.gov/tools.jsp) [17]. PTR was submitted as a query in DAVID. Homo sapiens was selected as the organism, and the selection criterion was $P < 0.05$.

Analysis of protein–protein interaction network and selection of hub gene

The protein–protein interaction (PPI) network of PTR was analyzed using STRING (https://string-db.org/) [18] version 11.5. The hub gene selection was performed using the CytoHubba plugin of Cytoscape, according to the highest degree score, and using default settings of the database based on a previous study.

Expression hub genes in breast cancer samples

Expression analyses of the hub genes in normal and tumor breast tissues were performed using GEPIA (http://gepia.cancer-pku.cn/) [19]. In brief, gene symbol was submitted into GEPIA using the BRCA datasets of the TCGA. The expression levels of hub genes in normal, tumor, and metastatic breast tissues were analyzed using TNMPlot (https://tnmplot.com/analysis/) [20]; those in several subtypes of breast cancer using TIMER2.0 (http://timer.cistrome.org/) [21, 22]; and those in HER2+ and HER2– breast cancer samples using Breast Cancer Gene-Expression Miner v4.8 (http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php?js=1) [23].

Correlation between the expression of PDCD1 and hub genes

Correlation analysis between the expression of *PDCD1* and hub genes was performed using TCGA breast cancer samples in GEPIA (http://gepia.cancer-pku.cn/) [19] and TIMER2.0 (http://timer.cistrome.org/) [21, 22]. In brief, gene symbol was submitted to GEPIA using TCGA samples of BRCA. Several parameters, such as HER2+ breast cancer samples and Spearman correlation values, were considered for the analysis in TIMER2.0. Statistical analysis was conducted using the Wilcoxon test (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001).

Prognostic value of PDCD1 and hub genes

Prognostic values of *PDCD1* and hub genes were analyzed using KMPlotter (https://kmplot.com/analysis/) [24]. In brief, gene symbols were submitted to KMPlotter, and selected parameters were chosen for instances HER2+ breast cancer, and the results are shown as relapse-free survival (RFS).

Genetic alteration analysis of PDCD1 and hub genes

The genetic alteration analysis of *PDCD1* and hub genes was performed using cBioportal (https://www.cbioportal.org/) [13]. In brief, *PDCD1* and hub genes were subjected to queries in cBioportal using the TCGA PanCancer Atlas study for Oncoprint and mutual exclusivity analysis.

ROC plot

The correlation of hub gene expression with sensitivity to trastuzumab in patients with breast cancer was analyzed using a receiver operating characteristic (ROC) plotter (http://www.rocplot.org) [25]. In brief, gene symbol was submitted to the ROC Plotter. Several parameters were selected, including ER status positive, RFS at five years, pathological complete response (PCR), and patients were treated with trastuzumab. Differences were considered statistically significant at P value < 0.05.

Correlation between PTR expression and infiltration of immune cells

The association between PTR gene expression and immune cell infiltration was investigated using TIMER2.0 (http://timer.cistrome.org/) [21, 22]. In brief, gene symbol was submitted to TIMER2.0. Several parameters were selected, such as HER2+ breast cancer samples and Spearman correlation values. Statistical analysis was conducted using the Wilcoxon signed-rank tests.

Results

Data mining

We obtained 1,092 coexpressed genes of *PDCD1*, including 1,085 and 7 genes with positive and negative Spearman correlation coefficients, respectively (Supplementary Table 1). From OMIM, we obtained 193

regulatory genes of trastuzumab resistance in breast cancer (Supplementary Table 2). The intersection of data from cBioportal and OMIM revealed 24 genes involved in PD-1 regulatory network responsible for the resistance of breast cancer cells to trastuzumab (Figure 1A, Supplementary Table 3).

GO and drug–gene association analysis

GO enrichment analysis of PTR was divided into three categories: biological processes, cellular components, and molecular functions. PTR was involved in several biological processes, such as response to stimulus, cell communication, and localization (Figure 1B, Supplementary Table 4). It is distributed in the membrane, endomembrane system, and vesicle. Additionally, PTR participates in protein binding, molecular transducer activity, and ion binding. Drug–gene association analysis revealed that PTR was associated with several monoclonal antibodies, such as sarilumab, palivizumab, and tositumomab (Figure 1C).

KEGG pathway enrichment analysis

KEGG pathway enrichment analysis of PTR revealed eight pathways regulated hsa05144:Malaria, hsa04660:T cell receptor signaling pathway, hsa05320:Autoimmune thyroid disease, hsa05150:Staphylococcus aureus infection, hsa05152:Tuberculosis, hsa05140:Leishmaniasis, hsa04666:Fc gamma R-mediated phagocytosis, and hsa05142:Chagas disease (American trypanosomiasis) (Supplementary Table 5).

PPI network analysis and hub gene selection

We successfully constructed the PPI network of PTR using STRING 11.5 with a confidence level of 0.4. This network consisted of 24 nodes, 47 edges, an average node degree of 3.92, a local clustering coefficient of 0.644, an expected number of edges of 6, and a PPI enrichment p-value of <1.0e-16 (Figure 1D). The hub gene selection

Table 1. Mutual Exclusivity Analysis of the Hub Genes

A	B	p-Value	Tendency
IL10	*PTPRC*	<0.001	Co-occurrence
PTPRC	*FCGR2B*	<0.001	Co-occurrence
IL10	*FCGR2B*	<0.001	Co-occurrence
PDCD1	*CTLA4*	0.006	Co-occurrence

Table 2. The ROC Plotter of the Correlation between *PDCD1, CTLA4, IL10, PTPRC, FCGR2B* and Relapse Free Survival (RFS), and Pathological Complete Response (PCR) of Trastuzumab-Treated Breast Cancer Patients.

Gene	RFS		PCR	
	R	P-value	R	P-value
PDCD1	0.517	0.42	0.619	1.90E-03
CTLA4	0.556	0.25	0.586	2.10E-02
IL10	0.507	0.47	0.539	0.18
PTPRC	NA	NA	NA	NA
FCGR2B	0.594	0.14	0.602	7.50E-03

Table 3. Correlation Analysis of Gene Expression of *PDCD1, CTLA4, IL10, PTPRC, FCGR2B* with the Levels of Immune Cells, as Analyzed Using TIMER.

Gene		Purity		B Cells		CD8		CD4		Neutrophil		Dendritic cells		Macrophage		Cancer Associated Fibroblast		Tregs		MDCs	
		Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P
PDCD1		-0.467	3.05E-05	0.758	1.30E-14	0.574	1.39E-07	0.477	2.30E-05	0.496	9.19E-06	0.752	2.79E-14	0.464	3.98E-05	-0.04	7.37E-01	0.252	3.27E-02	-0.59	4.92E-08
CTLA4		-0.504	5.54E-06	0.738	1.35E-13	0.5	7.67E-05	0.384	8.83E-04	0.586	6.55E-08	0.749	3.66E-14	0.513	3.98E-06	-0.208	7.98E-02	0.115	3.37E-01	-0.556	3.96E-07
IL10		-0.399	4.66E-04	0.552	5.01E-07	0.249	3.53E-02	0.293	1.25E-02	0.626	4.11E-09	0.578	1.07E-07	0.638	1.72E-09	-0.074	5.34E-01	0.041	7.33E-01	-0.572	1.49E-07
PTPRC		-0.556	3.21E-07	0.681	4.75E-11	0.37	1.30E-03	0.401	4.78E-04	0.68	5.03E-11	0.697	1.05E-11	0.545	7.36E-07	-0.152	2.01E-01	0.063	5.98E-01	-0.704	5.35E-12
FCGR2B		-0.453	5.72E-05	0.312	7.57E-03	-0.037	7.58E-01	0.065	5.86E-01	0.469	3.27E-05	0.326	5.21E-03	0.558	3.57E-07	0.104	3.83E-01	-0.018	8.79E-01	-0.379	1.04E-03

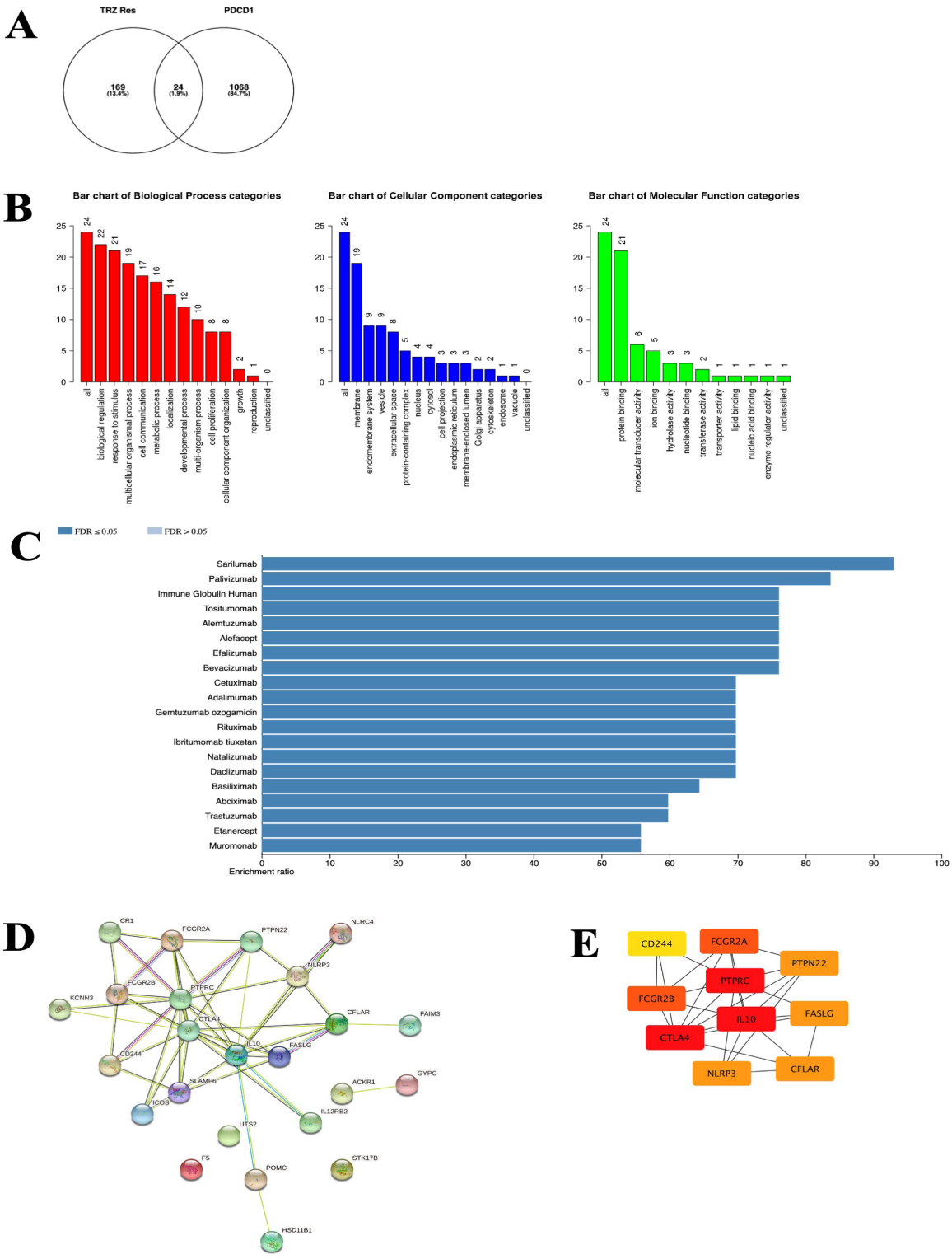

Figure 1. (A) Venn diagram of trastuzumab resistance and programmed cell death-1 (*PDCD1*) regulatory network, resulted in 24 genes which considered as genes involved in *PDCD1*-Trastuzumab Resistance regulatory network (PTR). (B) Gene ontology enrichment analysis of 24 PTR. (C) Drug–gene association analysis of the PTR. (D) Protein–protein interaction network of 24 PTR. (E) Top 10 of hub genes from PTR based on degree score.

using the CytoHubba plugin of Cytoscape yielded 10 genes with the highest degree scores, namely, cytotoxic T-lymphocyte–associated antigen 4 (*CTLA4*), protein tyrosine phosphatase receptor type C (*PTPRC*), interleukin (*IL)-10, FCGR2B, FCGR2A, PTPN22, CFLAR, NLRP3, FASLG,* and *CD244* (Figure 1E, Supplementary Table 6).

Expression hub genes in breast cancer samples

Gene expression analysis using the TCGA dataset in GEPIA showed that the mRNA levels of *PDCD1*, *CTLA4*, and *PTPRC* increased in patients with breast cancer (Figure 2A), whereas the mRNA levels of *IL10* and *FCGR2B* were not obvious. The mRNA levels of *PDCD1*, *CTLA4, PTPRC*, and *FCGR2B* were higher

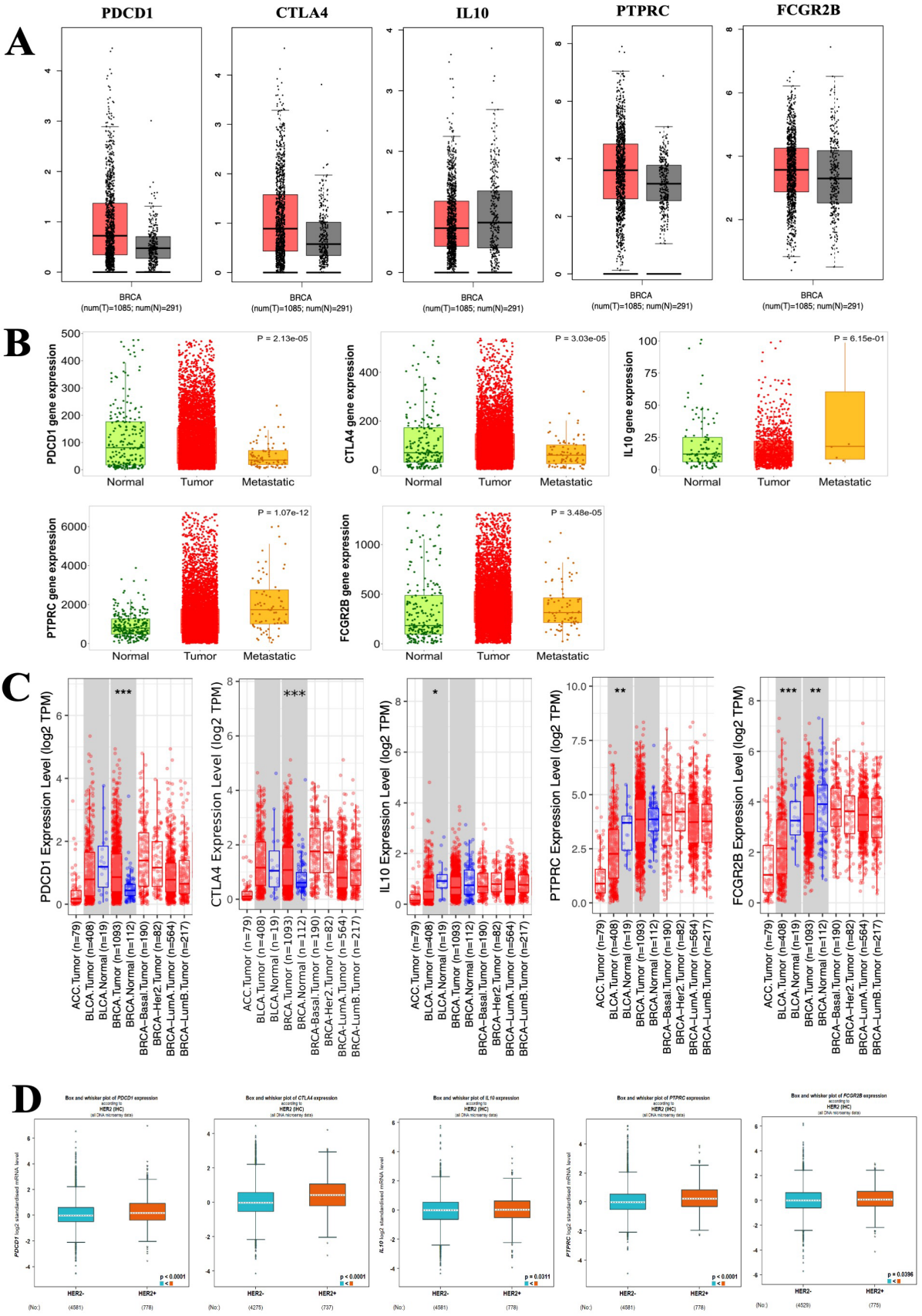

Figure 2. (A) mRNA expression of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* in normal and breast cancer tissues, as analyzed using GEPIA. (B) mRNA expression of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* in normal, breast cancer, and metastatic breast cancer tissues, as analyzed using TNMPlot. (C) mRNA expression of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* in normal and subtypes of breast cancer tissues, as analyzed using TIMER 2.0. (D) mRNA expression of PTR in HER2+ and HER2− breast cancer tissues, as analyzed using Breast Cancer Gene-Expression Miner v4.8.

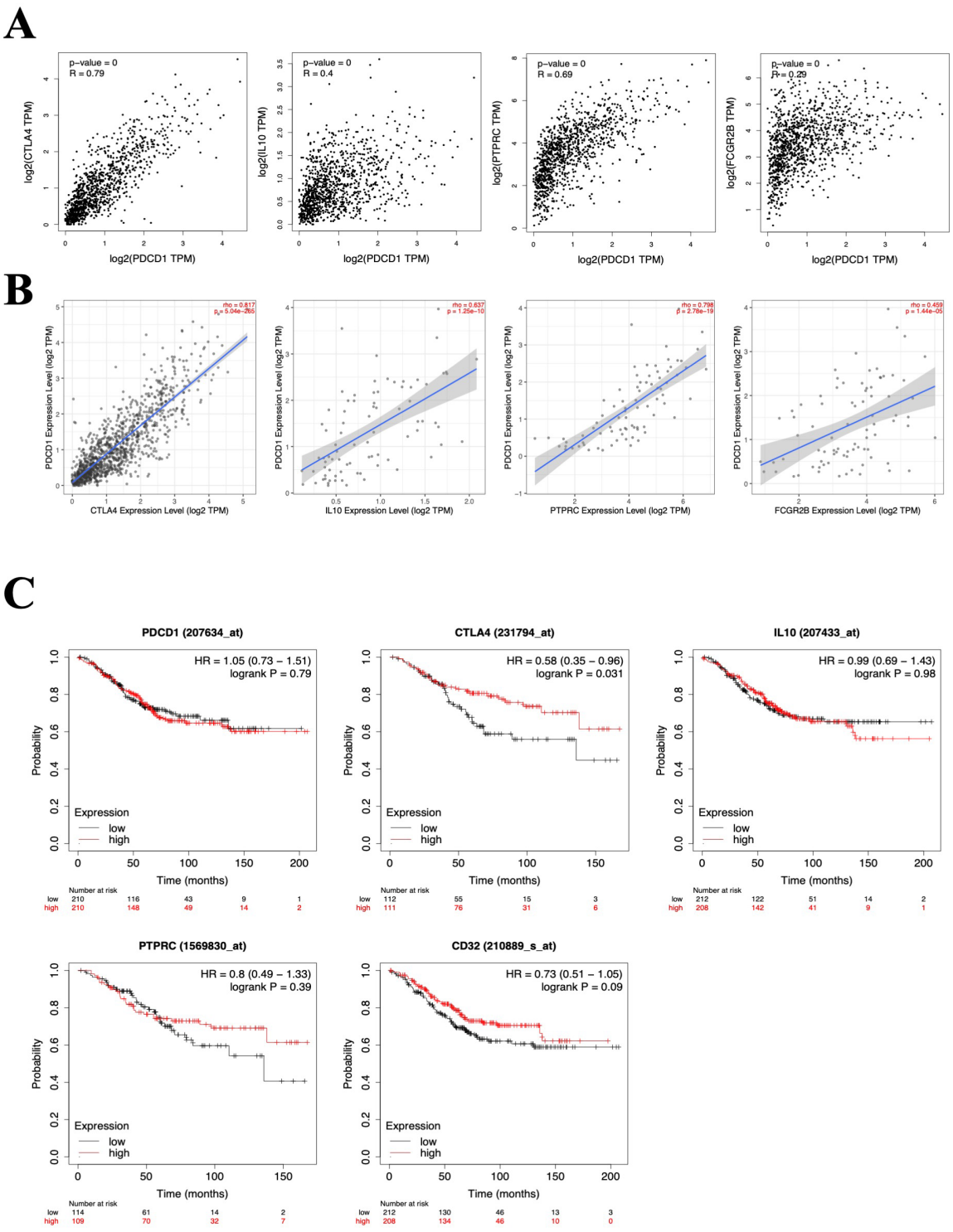

Figure 3. (A) Correlation of mRNA expression of *PDCD1* and *CTLA4, IL10, PTPRC, FCGR2B* in breast cancer samples, as analyzed using GEPIA. (B) Correlation of mRNA expression of *PDCD1* and *CTLA4, IL10, PTPRC, FCGR2B* in HER2+ breast cancer samples, as analyzed using TIMER. (C) Prognostic value related to the expression of PR in HER2+ breast cancer patients, as analyzed using KMPlotter.

in metastatic breast cancer cells than in breast cancer tissues (Figure 2B). In contrast, *IL10* levels were shown to be higher in metastatic breast cancer tissues. We also measured the mRNA levels in normal breast and HER2+ breast cancer tissues, and the results revealed that the levels of *PDCD1* and *CTLA4* were higher in HER2+ breast cancer tissues than in normal breast tissues (Figure 2C). The levels of *IL10, PTPRC*, and *FCGR2B* were not obvious between HER2+ and normal breast tissues. Additionally, we also checked the mRNA concentration

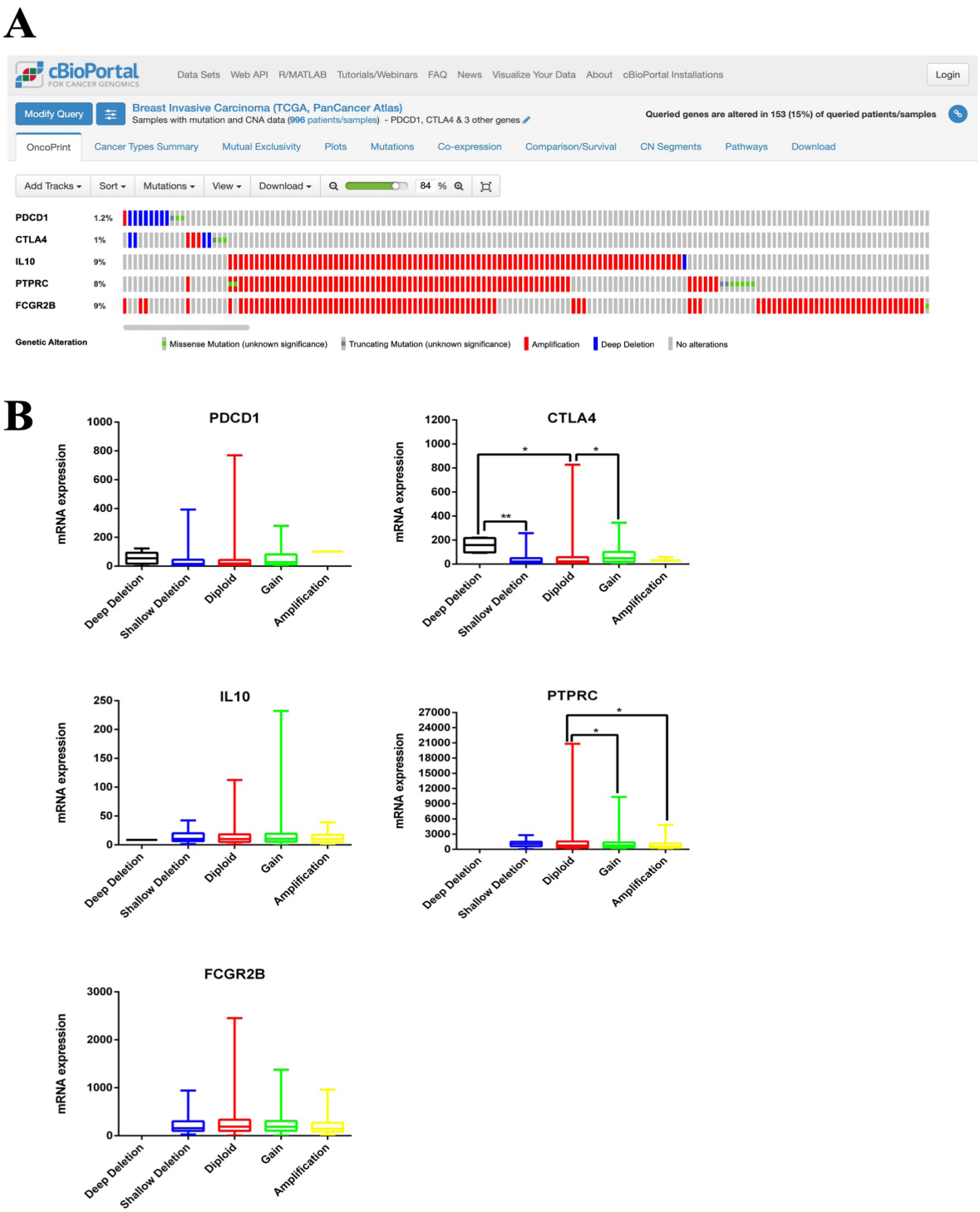

Figure 4. (A) Oncoprint analysis of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* in breast cancer samples from TCGA study. (B) Copy number alterations of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* across TCGA Pancancer study in the cBioportal database. Statistical analysis was performed using ANOVA continued with Tukey's multiple comparison test. *or ** indicates $p < 0.05$ or $p < 0.01$, respectively.

of hub genes and *PDCD1* in HER2+ and HER2−. The mRNA levels of *PDCD1*, *CTLA4, IL10, PTPRC*, and *FGCR2B* were significantly increased in HER2+ breast cancer patients (Figure 2D).

Correlation between PDCD1 expression and hub genes

Correlation analysis between the expression of *PDCD1* and hub genes in breast cancer and adjacent tissues using GEPIA showed that *CTLA4* (R = 0.79), *IL10* (R = 0.4), *PTPRC* (R = 0.69), and *FCGR2B* (R = 0.29) were directly correlated with *PDCD1* expression (Figure 3A). We also checked the correlation in HER2+ breast cancer cells using TIMER2.0, and the results showed that *CTLA4*, (R = 0.817), *IL10* (R = 0.637), *PTPRC* (R = 0.798), and *FCGR2B* (R = 0.459) were directly correlated with *PDCD1* expression (Figure 3B).

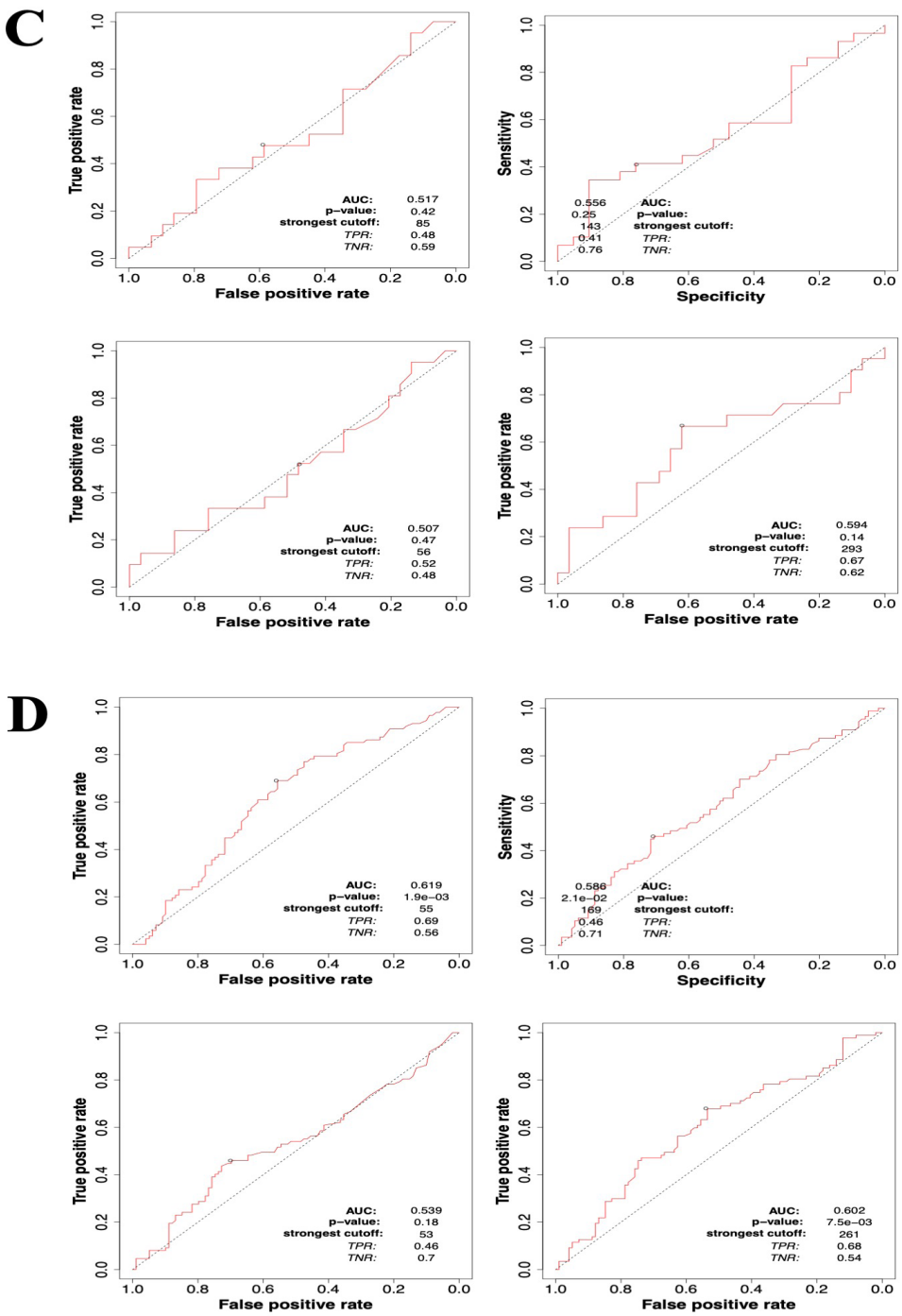

Figure 4. The ROC plotter of the correlation between *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* expression and trastuzumab sensitivity, as analyzed based on relapse-free survival (C) and pathological complete response (D).

Prognostic value of PDCD1 and hub genes

Prognostic value of *PDCD1* and hub genes using KMPlotter showed that high mRNA levels of *CTLA4* had a significantly better RFS than the opposite groups in patients with HER2+ breast cancer (Figure 3C). Additionally, the expression of *PDCD1*, *IL10, PTPRC,* and *FCGR2B* were not so obvious.

Genetic alteration analysis of PDCD1 and hub genes

We performed the genetic alteration analysis of *PDCD1* and hub genes using cBioportal using samples from the TCGA PanCancer Atlas study. Oncoprint analysis showed that the alterations occurred in 1%–9% samples, including *PDCD1* (1.2%), *CTLA4* (1%), *IL10* (9%), *PTPRC* (8%), and *FCGR2B* (9%) (Figure 4A). Most of the cases in genetic alterations occurred were amplification and deep deletion. Further mutual exclusivity analysis showed that four gene pairs co-occurred in mutation, including *IL10–PTPRC, PTPRC–FCGR2B,* and *IL10–FCGR2B* (Table 1). Analysis of copy number alterations revealed that the mRNA expression of *CTLA4* was higher in the case of gain than in the case of diploid

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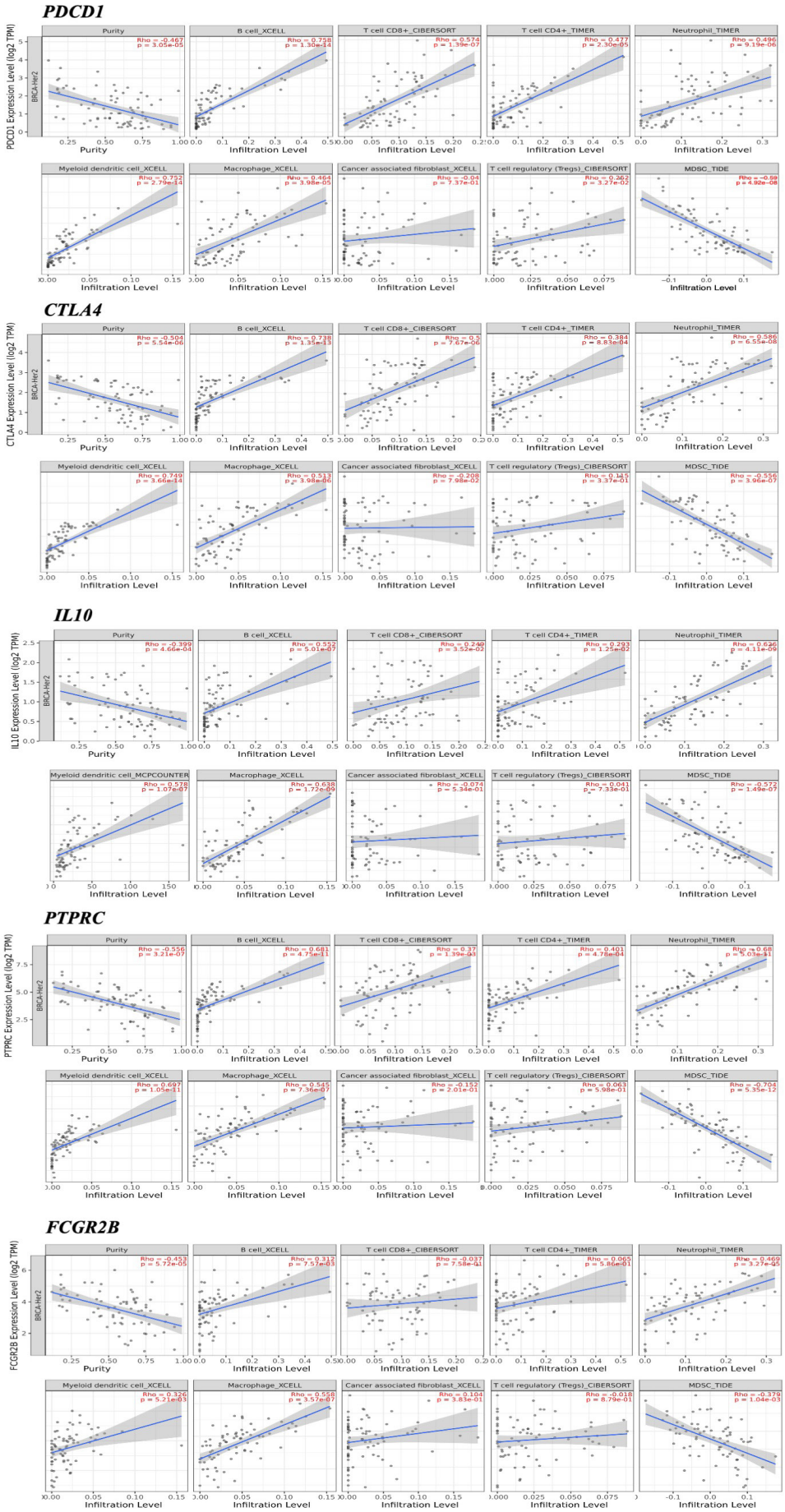

Figure 4. (E) Correlation analysis of gene expression of *PDCD1, CTLA4, IL10, PTPRC*, and *FCGR2B* with the levels of immune cells, as analyzed using TIMER 2.0.

and was higher in the case of deep deletion than in the case of shallow deletion (Figure 4B). Moreover, the mRNA levels of *PTPRC* were higher in cases of gain and amplification than in the case of diploid.

ROC Plot

The expression levels of *PDCD1*, *CTLA4, IL-10, PTPRC*, and *FCGR2B* did not correlate with the RFS of trastuzumab-treated patients with breast cancer (Table 2, Figure 4C). The expression levels of *PDCD1*, *CTLA4*, and *FCGR2B* had a moderately significant prognostic power correlation in the PCR of trastuzumab-treated patients with breast cancer, those with AUC values of 0.619, 0.586, and 0.602, respectively (Figure 4D).

Correlation between PTR expression and immune cells infiltration

TIMER 2.0 was used to investigate the relationship between PTR gene expression and infiltration of immune cells in HER2+ breast cancer cells. Purity was negatively correlated with the expression levels of *PDCD1* (r = −0.467, p = 3.05E-05), *CTLA4* (r = −0.504, p = 5.54E-06), *IL10* (r = −0.399, p = 4.66E-04), *PTPRC* (r = −0.556, p = 3.21E-07), and *FCGR2B* (r = −0.453, p = 5.72E-05) (Table 3, Figure 4B). B cells were positively correlated with the expression levels of *PDCD1* (r = 0.758, p = 1.30E-14), *CTLA4* (r = 0.738, p = 1.35E-13), *IL10* (r = 0.552, p = 5.01E-07), PTPRC (r = 0.681, p = 4.75E-11), and *FCGR2B* (r = 0.312, p = 7.57E-03). Neutrophils were positively correlated with the expression levels of *PDCD1* (r = 0.496, p = 9.19E-06), *CTLA4* (r = 0.586, p = 6.55E-08), *IL10* (r = 0.626, p = 4.11E-09), PTPRC (r = 0.68, p = 5.03E-11), and *FCGR2B* (r = 0.469, p = 3.27E-05). Dendritic cells were positively correlated with the expression levels of *PDCD1* (r = 0.752, p = 2.79E-14), *CTLA4* (r = 0.749, p = 3.66E-14), *IL10* (r = 0.578, p = 1.07E-07), PTPRC (r = 0.697, p = 1.05E-11), and *FCGR2B* (r = 0.326, p = 5.21E-03). Cancer-associated fibroblasts (CAFs) were negatively correlated with the expression levels of *PDCD1* (r = −0.04, p = 7.37E-01), *CTLA4* (r = −0.208, p = 7.98E-02), *IL10* (r = −0.074, p = 5.34E-01), and PTPRC (r = −0.152, p = 2.01E-01) and positively correlated with the expression of *FCGR2B* (r = 0.104, p = 3.83E-01). CD8 positively correlated with the expression levels of *PDCD1* (r = 0.574, p = 1.39E-07), *CTLA4* (r = 0.5, p = 7.67E-05), *IL10* (r = 0.249, p = 3.53E-02), PTPRC (r = 0.37, p = 1.30E-03), and *FCGR2B* (r = −0.037, = p7.58E-01). CD4 positively correlated with the expression levels of *PDCD1* (r = 0.477, p = 2.30E-05), *CTLA4* (r = 0.384, p = 8.83E-04), *IL10* (r = 0.293, p = 1.25E-02), PTPRC (r = 0.401, p = 4.78E-04), and *FCGR2B* (r = 0.065, 5.86E-01). Macrophages positively correlated with the expression levels of *PDCD1* (r = 0.464, p = 3.98E-05), *CTLA4* (r = 0.513, p = 3.98E-06), *IL10* (r = 0.638, p = 1.72E-09), PTPRC (r = 0.545, p = 7.36E-07), and *FCGR2B* (r = 0.558, p = 3.57E-07). Regulatory T cells (Tregs) positively correlated with the expression levels of *PDCD1* (r = 0.252, p = 3.27E-02), *CTLA4* (r = 0.115, p = 3.37E-01), *IL10* (r = 0.041, p = 7.33E-01), PTPRC (r = 0.063, p = 5.98E-01), and *FCGR2B* (r = -0.018, p = 8.79E-01). Myeloid dendritic cells (mDCs) were negatively correlated with the expression levels of *PDCD1* (r = −0.59, p = 4.92E-08), *CTLA4* (r = −0.556, p = 3.96E-07), *IL10* (r = −0.572, p = 1.49E-07), PTPRC (r = −0.704, p = 5.35E-12), *FCGR2B* (r = −0.379, p = 1.04E-03).

Discussion

Resistance to trastuzumab remains a significant hurdle for treating HER2+ breast cancer cells [26]. In this study, we explored the PD-1 regulatory network on breast cancer cell resistance to trastuzumab. HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy [27]. This study identified four genes associated with the *PDCD1* regulatory network in trastuzumab-resistant HER2+ breast cancer cells (PTR): *CTLA4*, *IL10*, *PTPRC,* and *FCGR2B*. PTRs were upregulated in patients with HER2+ breast cancer. *PDCD1* expression positively correlated with *CTLA4* and *PTPRC* with Spearman correlation coefficients of 0.817 and 0.789, respectively. The mRNA expression levels of *CTLA4* and *PTPRC* were higher in the cases of gain and amplification than in the case of diploid. The ROC plot showed that *PDCD1*, *CTLA4*, and *FCGR2B* had a moderately significant prognostic power correlation in the PCR of patients with trastuzumab-treated breast cancer.

One of the hallmarks of cancer is the resistance to immune therapy, which is triggered by extrinsic factors, including tumor-associated macrophages, myeloid-derived suppressor cells, and Tregs that generate immunosuppressive factors and secrete inhibitory ligands that interact with receptors on T cells, including PD-1 and CTLA-4 [28]. The binding of PD-1 and its ligand PD-LI activates negative regulators of T cell immune function and thus decreases the activation of the immune system [29]. Aberrant expression of PD-LI in the tumor microenvironment is involved in the activation of multiple oncogenic signaling by inflammatory factors, such as interferon gamma [30], especially in trastuzumab-resistant HER2+ breast cancer cells [31]. Inhibition of PD-1 and PD-LI interaction inhibits T cell response, and closure of this interaction has been shown for immunotherapy [32].

PD-1 inhibitors, such as nivolumab and pembrolizumab, have been authorized for non-small cell lung cancer and metastatic melanoma [33]. Moreover, trastuzumab increases the expression of PD-LI via activation of NFkB signaling. The activation of HER2 signaling also regulates trastuzumab efficacy and tumor-infiltrating immune cells by stimulating CCL2 and PD-LI in HER2+ breast cancer cells [27]. Bispecific antibodies that target PD-1 and HER-2 inhibit HER2+ metastatic breast cancer [3, 34]. Li et al. successfully overcome trastuzumab resistance in breast cancer cells by PD-1 blockade and using anti-HER2 chimeric antigen receptor (CAR) T cells [26]. A previous study showed that bispecific antibodies against ErbB2 and PD-L1 improve targeted anti-ErbB2 therapy [34]. Li et al. conducted a single and combination study of anti-HER2 CAR-T cells and anti-PD-1; they found that single anti-HER2 CAR shows beneficial effects on HER2+ breast cancer cells, and this effect is increased when it is combined with anti-PD-1 [26]. A case study by Wang

showed that PD-1 antibody camrelizumab increases the sensitivity to chemotherapy and trastuzumab in patients with HER2+ metastatic gallbladder cancer [35]. Another in vivo study showed that the combined treatment of PD-1-Vaxx combo HER-2 peptide vaccine (B-Vaxx) increases tumor growth suppression in CT26/HER2-xenografted colon cancer [36].

CTLA-4, also known as CD152 (cluster of differentiation 152), is a protein receptor that functions as an immune checkpoint and suppresses the immune response [37]. The inhibition of CTLA-4 and PD-1 leads to the activation of the immune system, and this strategy has been used for immunotherapy against non-small cell lung cancer and metastatic melanoma [33]. A combination of anti-PD-1 and anti-CTLA-4 improves the response rates of their single treatment [29]. A commercial CTLA-4 inhibitor, ipilimumab, has been authorized for treating metastatic melanoma [33]. Therapy using ipilimumab and pembrolizumab increases the survival of patients with melanoma [38]. Loi performed a clinical trial of combined trastuzumab and pembrolizumab in trastuzumab-resistant HER2+ breast cancer cells and found that the combined treatment benefit patients with PDL1+ trastuzumab-resistant HER2+ breast cancer cells [39]. A review article discussed the development of immunotherapy against HER2+ breast cancer cells using CTLA-4 inhibitors, anti-PD-1/PD-LI antibodies, and immune checkpoint inhibitors [40].

Cytokines are factors that influence cancer proliferation, survival, metastasis, and even resistance [41]. IL-10 is an immunoregulatory cytokine that acts as a poor prognostic cause and is involved in the breast cancer progression [41]. IL-10 induces immunosuppression and evades the tumor immune response; and also exerts proliferative and inhibitory effects on breast cancer cells [42].

PTPRC (or CD45) is a transmembrane glycoprotein found on practically all hematopoietic cells, except for mature erythrocytes that regulate T and B cell antigen receptor-mediated activation [43]. CD45 is the elusive JAK tyrosine phosphatase that inhibits the activation of cytokine receptor, which is implicated in hematopoietic cell antiviral immunity, proliferation, and differentiation [44]. CD45 phosphatase activity is overexpressed in leukemia and lymphoma patients [43]. Wei 2021 reported that PTPRC is a possible prognostic marker in lung adenocarcinoma because it participates in the regulation of the tumor microenvironment immunological state, which may alter the activity of T cells and other immune cells [45]. After chemoradiation therapy, *PTPRC*, which encodes CD45, is upregulated in leftover tumor tissues and thus has been linked to metastasis [46]. CD45 also promotes chemoradiation therapy resistance by activating Wnt/β-catenin pathway in colorectal cancer cells [46]. Mcnamara 2021 found that CD45 is a biomarker for stratification of response prediction to neoadjuvant therapy in HER2+ breast cancer [47]. However, the role of CD45 in the PD-1 regulatory network of trastuzumab resistance needs to be further clarified.

Therapy with monoclonal antibodies against HER2 trastuzumab mediates fragment crystallizable gamma receptor-dependent activities, such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis, which are innate immune mechanisms of cancer cell elimination, as part of their mechanism of action [48]. *FGCR2B* encodes Fc γ receptor IIB (FcγRIIB), an inhibitory molecule that can reduce antibody immunotherapy efficacy [49]. FcγRIIB possesses a cell intrinsic co-inhibitory function in regulating CD8+ T cell immunity [50]. The higher the *FCGR2B* expression, the shorter is the progression-free survival in diffuse large B cell lymphoma patients [49]. Additionally, *FCGR2B* expression has been linked to tumor FcγRIIB expression and to a shorter pathological free survival. A multiomics study by Zhang 2019 demonstrated the association of *PD-1, PD-LI, CTLA-4*, and *FCGR2B* with immunosuppressive function in glioblastoma multiforme [51]. Another bioinformatics study showed that *FCGR2B* is associated with radioresistance in glioblastoma [52]. However, the significance of FcγRIIB in the PD-1 regulatory network of trastuzumab resistance remains unclear and deserves further investigations.

Failure to induce immune-mediated cellular response to eradicate cancer cells is a causative mechanism of breast cancer resistance to trastuzumab [8]. This study showed that PTR expression generally showed a direct correlation with infiltration of B cell, CD8+ and CD4+ cells, neutrophils, dendritic cells, macrophages, and Tregs but an inverse correlation with purity, CAF, and mDCs. ADCC involves interacting FCGR with trastuzumab and T cells [48]. Treatment with trastuzumab induces the expression of PD-LI through NFkB signaling. [33]. IL-10 levels are higher in HER2+ breast cancer cells than in those with HER2− [53]. A metabolomics study by Vignoli, [54] showed that IL-10 levels are a prognostic of relapse in HER2+ breast cancer patients subjected to neoadjuvant targeted chemotherapy [54]. IL-10 is upregulated by PD-LI in hepatocellular carcinoma cells [55]. CTLA-4 and PD-1 immune checkpoints are negative regulators of the T cell immune function. CTLA-4 regulates T cell proliferation in the early immune response in lymph nodes, while PD-1 suppresses T cell proliferation later in peripheral tissues [33]. A recent study has shown that CD45 regulates Wnt/ β-catenin pathway in colorectal cancer cells [46]. However, the role of CD45 in trastuzumab resistance remains uncertain.

The findings of this study are valuable for elucidating the mechanism of breast cancer cell resistance to trastuzumab, specifically the regulatory network. Moreover, the findings of this study may facilitate the development of therapeutic drugs for overcoming trastuzumab resistance in breast cancer cells. Further studies using cell lines, animals, or even clinical trials are warranted to validate the findings of this study.

In conclusion, this study revealed four genes (*CTLA-4, IL-10, PTPRC*, and *FCGR2B*) associated with the *PDCD1* regulatory network in trastuzumab-resistant HER2+ breast cancer cells (PTR). Further studies are needed to develop a therapeutic target against trastuzumab resistance in HER2+ breast cancer.

Author Contribution Statement

The author, AH, made significant contributions to the study's idea and design, data gathering, analysis, interpretation, article drafting and revision, and final approval of the published edition. HP participated in data analysis and paper drafting.

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. Supplementary files are available in http://ugm.id/*PDCD1*

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Ethics issues and approval

Not applicable.

Conflict of interests

The authors declare that they have no competing interests.

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