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Application of Bioinformatics Analysis to Identify Important Pathways and Hub Genes in Breast Cancer Affected by HER-2

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ABSTRACT

Background: Human epidermal growth factor receptor 2 (HER-2) is used as a marker for the diagnosis and prognosis of breast cancer. However, the molecular mechanisms involving HER2 in breast cancer require further study.
Objective: Herein, we used bioinformatics approach to identify important pathways and hub genes in breast cancer affected by HER-2. The results showed that HER-2 is highly expressed in ovarian cancer and is closely related to the overall survival and progression-free survival of breast cancer.
Methods: A total 3014 downregulated genes and 4121 upregulated genes were identified under Gene Expression Omnibus (GEO) database with GEO2R tool.
Results: Among them, top 10 hub genes including CCNB1, KIF11, BUB1B, TOP2A, ASPM, MAD2L1, BUB1, RRM2, EGFR, and FN1 demonstrated by connectivity degree in the protein-protein interaction (PPI) network were screen out. In Kaplan–Meier plotter survival analysis, the overexpression of CCNB1, EGFR, MAD2L1, ASPM, and RRM2 were shown to be associated with an unfavorable prognosis in HER-2 positive breast cancer patients.
Conclusion: In conclusion, we have identified important signaling pathways involving HER-2 that affect breast cancer. These findings could provide new insights outlining mechanisms involving HER-2 gene expression in breast cancer and provides a rational for novel treatment of breast cancer.

Keywords: Bioinformatics analysis, HER-2, breast cancer, hub genes.

INTRODUCTION

Breast cancer (BC) is one of the most commonly diagnosed malignancies and a major cause of cancer mortality in women worldwide [1]. For the year 2020, it is estimated that in the Indonesia approximately 198,670 female patients would be diagnosed with BC and 41,760 would die from it [2,3]. Human epidermal growth factor receptor 2 (HER-2) positive breast cancer is caused by the amplification of the ERBB2/NEU receptor tyrosine kinase and represent approximately 20% of breast carcinomas [4]. HER-2 overexpression is related with an increased risk of disease recurrence and death in this breast cancer subtype [5,6], so patients with HER-2 positive breast cancer are treated with chemotherapy plus anti-HER2 inhibitors. Despite its beneficial effects, long-term treatment will produce drug resistance to the selected target [7]. Thus, the mechanisms underlying occurrence and development of HER-2 positive breast cancer need to be further clarified, and will provide important guidance for early diagnosis, improvement of prognosis, and more precise treatment of HER-2 positive breast cancer.

In the breast cancer, HER-2 may affect the oncogenesis of breast cancer through the PI3K/AKT signaling pathway [8]. In addition, studies have shown that HER-2 can influence the progression of cancer through a variety of genes or signaling pathways. For example, in the study of breast cancer,

HER-2 may affect the viability and chemoresistance of breast cancer cells in vitro by regulating the expression of the insulin-like growth factor 1 receptor (IGF1-R) Pathway[9–11]. Previous studies have shown that the HER-2 protein is highly expressed in breast cancer tissues and can regulate the metastasis and invasion of breast cancer through AKT/JNK/EMT pathway[12–14]. However, the signaling pathways, functional pathways, and key genes involved in HER-2 regulation of breast cancer need to be further defined.

We used bioinformatics analysis to study the specific mechanisms induced by HER-2 in regulating the process of breast cancer. In general, our systematic analysis will further elucidate the specific role played by HER-2 in the pathogenesis of HER-2 of breast cancer at the molecular level. Our findings will provide important guidance for the early diagnosis, improvement of prognosis, and precise treatment of ovarian cancer.

METHODS

Datasets and Identification of Differentially Expressed Genes

We set |log fold change (FC)|≧0.5, Q-value < 0.01 to identify differentially expressed genes (DEGs) between HER-2 positive breast cancer group and normal tissue samples. The gene expression profiles analyzed in this study were obtained from the GEO (The Gene Expression Omnibus) database (https://www.ncbi.nlm.nih.gov/geo/). The gene expression profiles GSE29431 was chosen of which all expression profiles were based on GLP570 platform. The GSE29431 datasets includes 12 normal tissues samples and 46 HER-2 positive breast cancer.

Data processing of DEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is an online tool to screen genes that are differentially expressed across different groups of samples. The raw microarray data files between HER-2 positive breast cancer and normal breast tissues were subsequently conducted by GEO2R. The adjusted P value and |logFC| were carried out for each dataset, with adjusted P<.01 and |logFC| ≥2.0 were considered as DEGs [15,16].

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were used [17], and followed by the Database for Annotation, Visualization and Integrated Discovery (David, http://david.abcc.ncifcrf.gov/) online tool to perform enrichment analysis, to calculate the p-value, and to perform FDR correction on p-value. P-values ≤ 0.05 and gene counts >5 were considered significantly enriched [18].

Protein–Protein Interaction Network Construction

We use the online database STRING to construct the Protein–protein interaction (PPI) network of DEGs, followed by the MCODE plug-in of Cytoscape software to perform module analysis on the constructed PPI network (node score cut-off = 0.2; max. depth = 100; k-core = 10). The CytoHubba plug-in was used for hub gene analysis was used for gene sequencing[19,20].

RESULTS

HER-2 was differentially expressed in breast cancer and was related to prognosis

In order to explore the potential significance of HER-2 in ovarian cancer, we used the online website UALCAN to analyze the pan-cancer expression of HER-2 and its expression in breast cancer

of different grades, stages, and ages. The analysis results showed that compared with other cancers, HER-2 was highly expressed in breast cancer (BRCA) (Figure 1A). HER-2 protein expression in breast cancer tissues was significantly higher than that in normal tissues (Figure 1B), and was found to be positively correlated with clinical stage and age of breast cancer (Figure 1C-D). Interestingly, the HER-2 highly express only in HER-2 positive breast cancer (Figure 1E). We used Kaplan-Meier Plotter online software to analyze the correlation between HER-2 and prognosis, and the results indicated that HER-2 was correlated with worse survival: log-rank p = 0.58 for overall survival (Figure 1F).

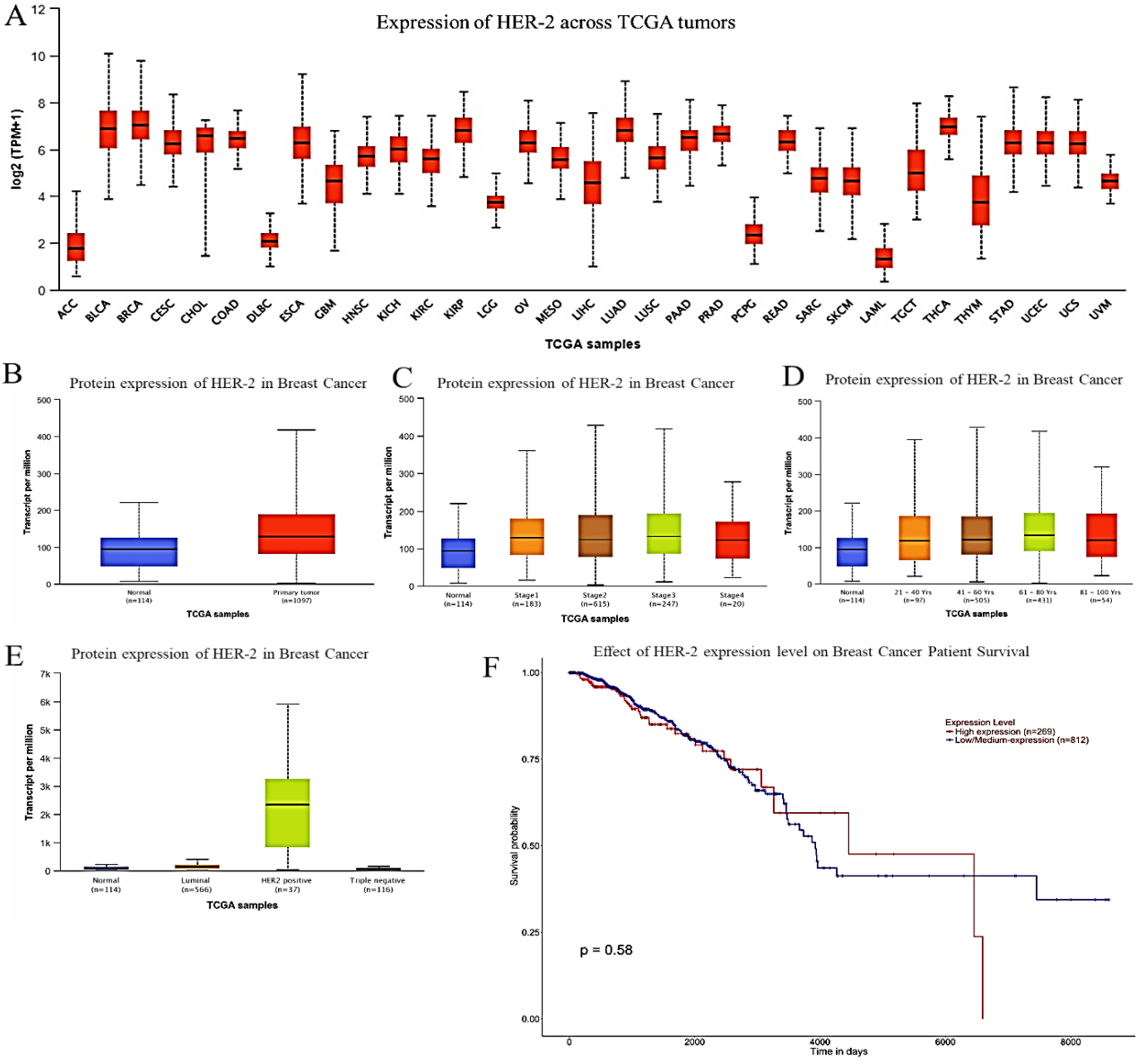

Figure 1. Bioinformatics analysis of the clinical significance of HER-2 in breast cancer (A) The expression level of HER-2 mRNA in different cancers was analyzed through the UALCAN website. The expression of HER-2 protein in breast cancer tissues and normal ovarian tissues (B), ovarian cancers stratified by different stages (C), age (D), and major subclass (E) were analyzed through the UALCAN website. Kaplan-Meier curves showing the correlation between the expression of HER-2 and overall survival (F) of patients with breast cancer.

Identification of DEGs, functional and pathway enrichment analyses

Based on GEO2R analysis from GSE29431, we obtained 3014 downregulated genes and 4121 upregulated genes compare with the control group. The volcano map showed that there was a large number of deferentially expressed genes in the two set of samples (Figure 2). The top 250 DEGs were uploaded to DAVID to identify significant GO categories and KEGG pathways. The results of GO analysis demonstrated that DEGs were markedly enriched in BP, including signal transduction, cell differentiation, cell migration, autophagy, mitotic cell cycle, PI3K signaling, and cell adhesion. Go CC analysis also showed that DEGs were enriched in cell surface, focal adhesion, and actin cytoskeleton. As for MF analysis, DEGs were significantly enriched in protein binding and actin filament binding. In addition, the results of KEGG pathway analysis indicated that DEGs were mainly enriched in AMPK signaling pathway Rap1 signaling pathway, and PPAR signaling pathway (Table 1).

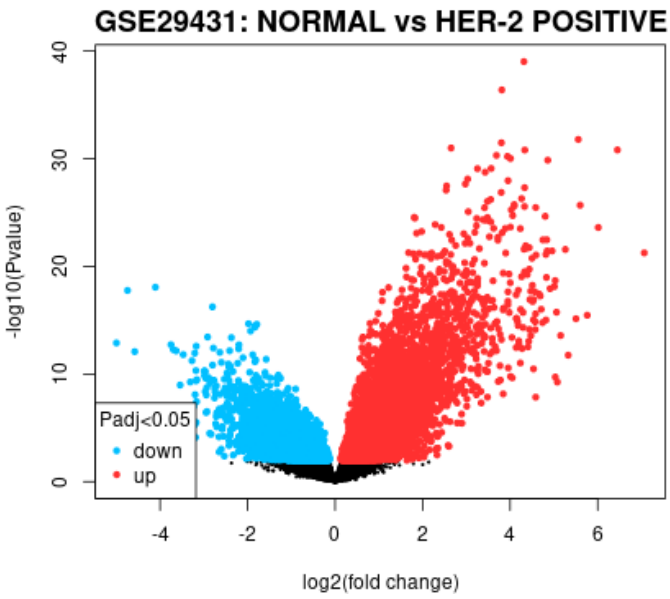

Figure 2. Volcano plot of DEGs in the HER-2 positive expression and normal group.

Differential Gene Protein-Protein Interaction (PPI) Network Construction and Core Gene Analysis

In total the 2000 top DEGs were imported into the STRING website and PPI network construction was carried out with a combined score >0.9 defines as significant. The results included 1255 nodes and 2538 edges were evaluated in the PPI network (Figure 3A). The degree score of Cytoscape software was used for further analysis, and the most important module was selected for subsequent analysis (Figure 3B). The top ten genes demonstrated by connectivity degree in the PPI network were CCNB1, KIF11, BUB1B, TOP2A, ASPM, MAD2L1, BUB1, RRM2, EGFR, and FN1 (Table 2). The main biological process involved included cell-cell adhesion, regulation of cell growth, wound healing, response to the stimulus, and regulation of cell growth.

Survival analysis of the identified hub genes

To evaluate the prognostic roles of the ten potential hub genes with HER-2 positive breast cancer, the Kaplan–Meier plotter bioinformatics analysis platform was applied. A total 882 HER-2 positive breast cancer patients were available for the analysis of relapse free survival (RFS) and overall survival (OS). We found that the higher expression of CCNB1, EGFR, MAD2L1, ASPM, and RRM2 reduce the survival time of HER-2 positive breast cancer patient (Figure 4).

Table 1. Gene ontology and KEGG pathway analysis of Top 250 DEGs associated with HER-2 positive breast cancer

Category	Function/Pathway	Count	*P* value
KEGG_PATHWAY	AMPK Signaling pathway	8	1.8E-4
KEGG_PATHWAY	Rap1 signaling pathway	6	5.8E-2
KEGG_PATHWAY	PPAR signaling pathway	6	3.9E-2
GEOTERM_BP_DIRECT	Signal transduction	18	1.1E-2
GEOTERM_BP_DIRECT	Cell differentiation	13	5.3E-2
GEOTERM_BP_DIRECT	Positive regulation of cell migration	7	1.5E-2
GEOTERM_BP_DIRECT	Negative regulation of autophagy	6	7.1E-3
GEOTERM_BP_DIRECT	negative regulation of G1/S transition of mitotic cell cycle	6	1.2E-2
GEOTERM_BP_DIRECT	phosphatidylinositol 3-kinase signaling	6	3.5E-2
GEOTERM_BP_DIRECT	positive regulation of cell adhesion	6	6.1E-2
GEOTERM_CC_DIRECT	Cell surface	12	8.8E-2
GEOTERM_CC_DIRECT	Focal adhesion	8	4.6E-2
GEOTERM_CC_DIRECT	Actin cytoskeleton	6	4.8E-2
GEOTERM_MF_DIRECT	Protein binding	108	6.7E-2
GEOTERM_MF_DIRECT	Actin filament binding	7	2.3E-2

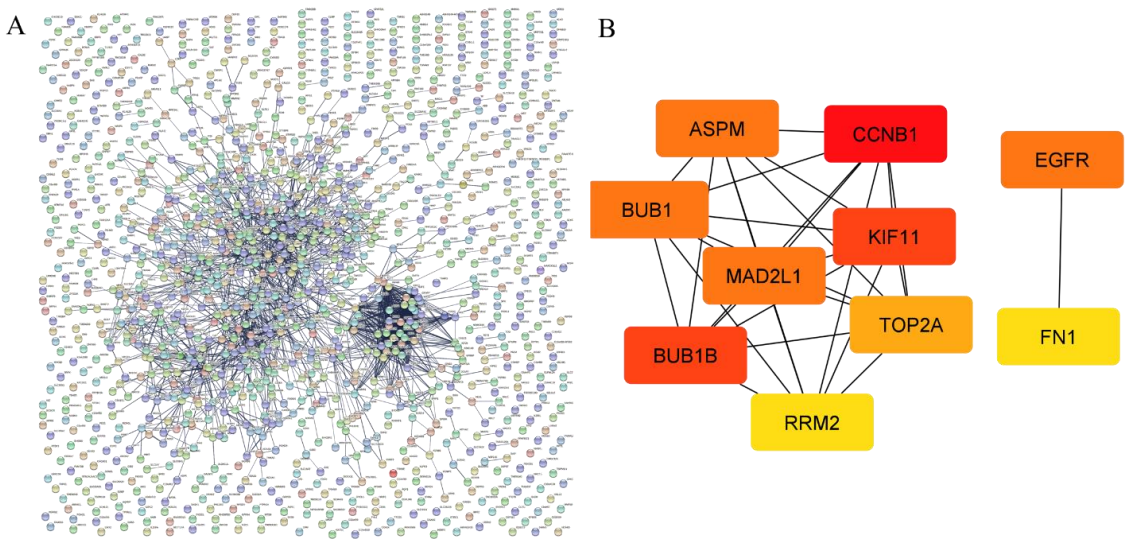

Figure 3. Differential gene expression PPI network construction and module analysis. (A) The PPI network of DEGs constructed using the STRING online database. The nodes represent proteins, and the edges represent protein interactions. (B) Module analysis using degree score of Cytoscape software. DEGs: differentially expressed genes.

DISCUSSION

Breast cancer is a heterogeneous disease in which the biological features and clinical behaviors vary from each subtype. HER-2 positive breast cancer is caused by the amplification of the ERBB2/NEU receptor and associated with an increased risk of disease recurrence and death [5]. Despite advances in current therapeutics such as anti-HER2 therapy, relapse or metastasis still occur after adjuvant treatment. Further understanding in the specific mechanisms induced by HER-2 in regulating

the process of breast cancer could offer a great number of potential clues in developing novel therapeutic agents.

In this study we used bioinformatic analysis to analyzed the effects of HER-2 on the signaling pathways, molecular functions, and the associated biological processes, and constructed a PPI network of DEGs and performed module analysis. In this study we found gene expression profiling of HER-2 through GEO databases to identify potential key genes related with HER-2 positive breast cancer. DEGs between HER-2 positive breast cancer and normal breast tissues were conducted by GEO2R, 3014 downregulated genes and 4121 upregulated genes were identified in total. These DEGs were shown to be mostly involved in the signal transduction, cell differentiation, cell migration, autophagy, mitotic cell cycle, PI3K signaling, and cell adhesion for GO BP term analysis and conformed our knowledge that these factors were of vital importance for tumor development and progression [21,22]. In this study we also found that DEGs enriched in focal adhesion, this resulted supported previous study that targeting focal adhesion kinase could improve trastuzumab response and might be an effective measure to overcome trastuzumab resistance in HER-2 positive breast cancer [23,24]. Moreover, the DEGs were found significantly enriched in KEGG pathways of AMPK signaling pathway Rap1 signaling pathway, and PPAR signaling pathway. PPAR signaling pathway was indicated to be a potential predictor of neoadjuvant chemotherapy response in breast cancer [25–27]. These signaling also played important roles in regulating the cancer process. In addition, AMPK was found dysfunctional in breast cancer, with the reduced signaling via the AMPK pathway was correlated with a higher axillary node metastasis of breast cancer through EMT transcription factor Snail1 [28,29]. The Rap1 signaling has been implicated in the regulation of cell proliferation and migration through regulation of integrin- or cadherin-mediated cell adhesion, expression levels of matrix metalloproteinase and cytoskeletal changes [30,31].

Table 2. Top ten genes demonstrated by connectivity degree in the PPI network

Gene symbol	Gene title	Degree
CCNB1	Cyclin B1	16
KIF11	Kinesin Family Member 11	15
BUB1B	BUB1 Mitotic Checkpoint Serine/Threonine Kinase B	14
EGFR	Epidermal Growth Factor Receptor	11
MAD2L1	MAD2 mitotic arrest deficient-like 1	10
ASPM	Assembly Factor For Spindle Microtubules	10
BUB1	Mitotic Checkpoint Serine/Threonine Kinase	9
TOP2A	DNA Topoisomerase II Alpha	8
FN1	Fibronectin 1	7
RRM2	Ribonucleotide Reductase Regulatory Subunit M2	7

PPI network and module analysis was also conducted to evaluated the association of the DEGs, 10 hub genes were revealed including CCNB1, KIF11, BUB1B, TOP2A, ASPM, MAD2L1, BUB1, RRM2, EGFR, and FN1. Despite there were more downregulated DEGs identified, all of these genes were found to be upregulated in HER-2 positive breast cancer. In the Kaplan-Meier plotter bioinformatics analysis, higher expression of CCNB1, EGFR, MAD2L1, ASPM, and RRM2 were indicated to be an unfavorable prognostic factor for HER-2 positive breast cancer patients. CCNB1 is important regulatory protein in the MAPK signaling pathway. CCNB1 is well known for its critical role in regulating Cyclin-dependent kinase 1 (Cdk1), which initiates the process from G2 phase to mitosis [32]. Overexpression of CCNB1 is indicated to be associated with aggressive phenotype and poor prognosis for breast cancer [33]. Overexpression of HER-2 correlated with EGFR expression leading to

metastatic breast cancer [34]. On the other hand, MAD2L1 as a component of spindle checkpoint, plays an essential role in supervising chromosomal segregation during mitosis. Previous study reported that higher expression of MAD2L1 in breast cancer associated with malignant progression and poor disease-free survival [35]. Abnormal spindle-like microcephaly associated gene (ASPM) is a regulator of Wnt and stemness in pancreatic adenocarcinoma which as a Wnt associated marker, it is not only could predict survival time but also could become a target therapy [36,37]. In addition, RRM2 is a key gene in pyrimidine metabolism and has been proved to be highly up-regulated in breast cancer patients. Relevant studies also suggested RRM2 as a prominent marker for breast cancer metastasis [38].

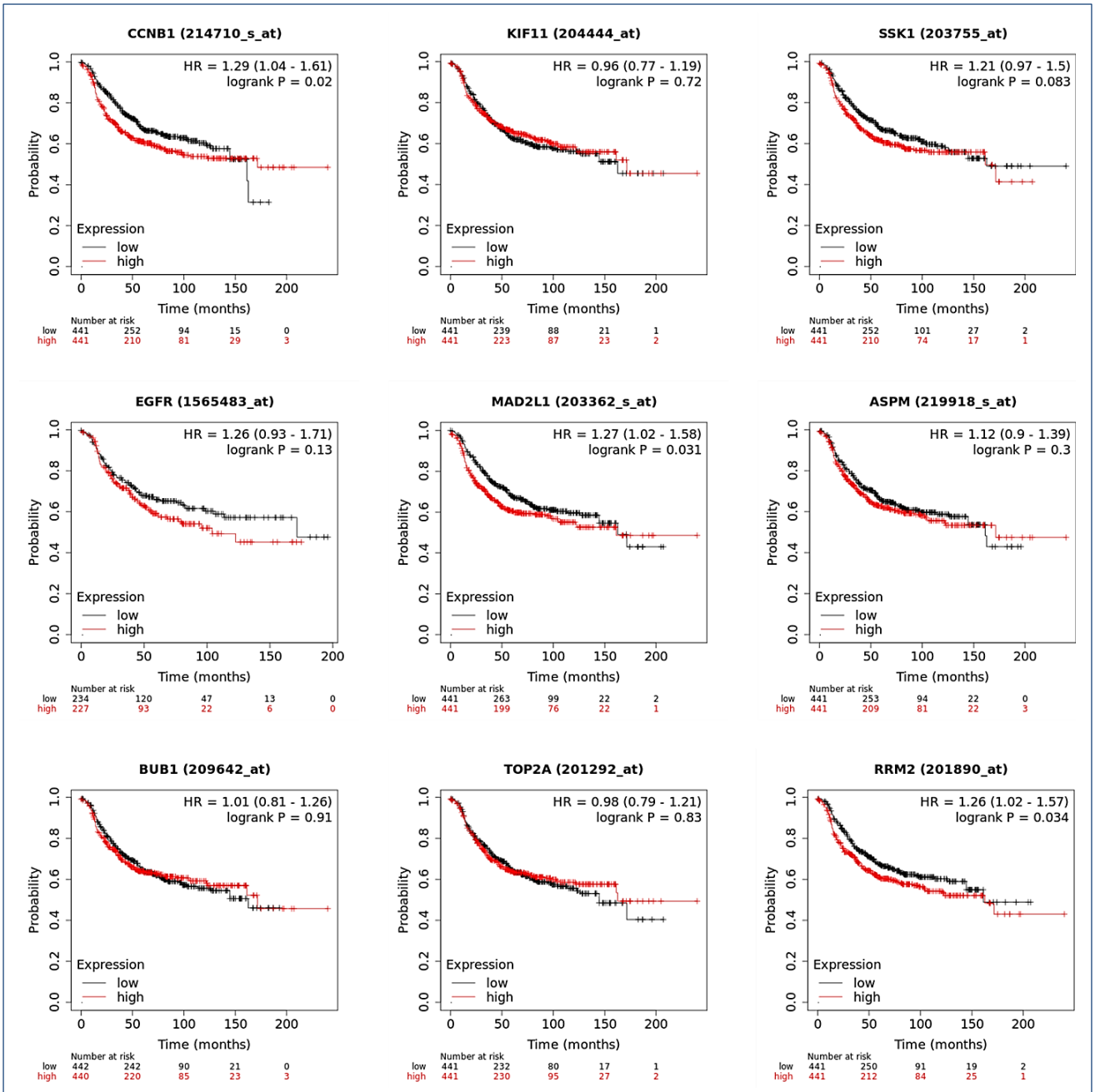

Figure 4. Kaplan–Meier survival analysis for the 10 potential hub genes in HER-2 positive breast cancer patients

CONCLUSION

In conclusion, we conducted a comprehensive bioinformatics analysis and revealed several potential target genes and pathways which might impact the oncogenesis and progression of HER-2 positive breast cancer. Our research not only helps to further enrich the possible involvement of HER-2 in the occurrence and development of breast cancer, especially the regulatory mechanisms of migration and invasion but also provides a theoretical rationale for establishing HER-2 as a suitable target for treatment and intervention of advanced breast cancer.

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AUTHOR CONTRIBUTION

NDA has made significant contribution to the concept and design, data acquisition, data analysis and supervised the project. AT has made significant contributions to concept and design, data analysis dan drafting of this manuscript. DH and MZ strictly revised the important knowledge content of the article and provided technical support. BC and BW provided technical support.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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