Original Article

Identification of key genes in HER2-positive breast cancer with brain metastasis via bioinformatics methods

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Background: Brain metastasis (BM) represents one of the most common advanced disease states in breast cancer (BC), especially in human epidermal growth factor receptor 2 (HER2)-positive BC, and is associated with poor survival outcomes.

Methods: In this study, in-depth analysis of the microarray data from the GSE43837 dataset with 19 BM samples of HER2-positive BC patients and 19 HER2-positive nonmetastatic primary BC samples was conducted. The differentially expressed genes (DEGs) between BM and primary BC samples were identified and function enrichment analysis of the DEGs was conducted to identify potential biological functions. The hub genes were identified by constructing the protein-protein interaction (PPI) network using STRING and Cytoscape. UALCAN and Kaplan-Meier plotter online tools were used to verify the clinical roles of the hub DEGs in HER2-positive BC with BM (BCBM).

Results: A total of 1,056 DEGs including 767 downregulated and 289 upregulated genes were identified by comparing the microarray data of the HER2-positive BM and primary BC samples. Functional enrichment analysis demonstrated that the DEGs were mainly enriched in pathways related to extracellular matrix (ECM) organization, cell adhesion, and collagen fibril organization. PPI network analysis identified 14 hub genes. Among these, *CD44*, *COL1A2*, *MMP14*, *POSTN*, and *SOX9* were associated with the survival outcomes of HER2-positive patients.

Conclusions: In summary, 5 BM-specific hub genes were identified in the study; those are potential prognostic biomarkers and therapeutic targets for HER2-positive BCBM patients. However, further investigations are necessary to unravel the mechanisms by which these 5 hub genes regulate BM in HER2-positive BC.

Keywords: Bioinformatics; differentially expressed genes (DEGs); human epidermal growth factor receptor 2-positive breast cancer (HER2-positive BC); brain metastasis (BM); survival

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Introduction

According to the International Agency for Research on Cancer, breast cancer (BC) is the most diagnosed malignancy worldwide (1). Brain metastasis (BM) is commonly observed in advanced BC patients with around 30% to 50% of metastatic BC (MBC) patients developing BM (2,3). The risk of BM as the first site of metastasis is low in stage I–II BC patients (4,5), but significantly increases in BC patients with stage III disease (6,7). In BC, 50% of human epidermal growth factor receptor 2 (HER2)-positive BC patients, 25% to 46% of triple-negative BC (TNBC) patients, and 10–15% of estrogen receptor (ER)-positive HER2-negative BC patients develop BM during their lifetime (8-11).

Although BC with BM (BCBM) patients can benefit from local treatments such as stereotactic radiosurgery (SRS), surgery, and to a lesser extent whole-brain radiation therapy (WBRT), the median survival time of patients with BM is only 3 to 27 months (12-14). Advancements in systemic management developed since the last two decades, which have improved outcomes of MBC patients, and the current median survival time of HER2-positive MBC patients is 5 years (15,16). The poor efficacy of anti-cancer drugs against metastases in the central nervous system (CNS) is mainly attributed to the blood–brain barrier, which prevents significant concentration of high molecular-weight drugs entering the brain tissues (17). Progressive CNS disease accounts for the mortality of 50% of HER2-positive BCBM patients (18). Whole exome sequencing of 86 matched BMs, primary tumors, and normal tissues showed that clinically relevant genomic characteristics were significantly different in 53% of paired brain metastatic samples and primary BC sample (19). Therefore, understanding the precise molecular mechanisms underlying BMs in HER2-positive BC patients is critical in developing effective BM-specific treatment strategies.

In this study, the microarray data of the HER2-positive primary BC tissues and HER2-positive BM tissues of BC patients from the Gene Expression Omnibus (GEO) database were analyzed to identify candidate BM-related biomarker genes that are associated with the survival outcomes of HER2-positive BCBM patients. We present this article in accordance with the STREGA reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2715/rc).

Highlight box

Key findings

- *CD44*, *COL1A2*, *MMP14*, *POSTN*, and *SOX9* were HER2-positive BCBM associated genes, and were found to be related to the survival outcomes of HER2-positive patients.

What is known and what is new?

- HER2-positive BC is one of the most common BC with BM. Some BMs and paired primary BC shows different genomic characteristics, and these differences shown on genes can be applied to modulate the management of BCBM.
- HER2-positive BCBM associated genes were screened and their clinical significance in BC was verified.

What is the implication, and what should change now?

- *CD44*, *COL1A2*, *MMP14*, *POSTN*, and *SOX9* are HER2-positive BCBM associated gene, and they are potential therapeutic targets. Further investigations are necessary to unravel the mechanisms.

Methods

Microarray data

The GEO database (https://www.ncbi.nlm.nih.gov/geo) is a public functional genomics data repository of the National Center for Biotechnology Information (NCBI) (20). The gene expression data of 19 BM tissues of HER2-positive BC patients and 19 HER2-positive non-metastatic primary BC samples were downloaded from the GSE43837 dataset (21). The probes in the Affymetrix Human X3P Array were converted into the corresponding gene symbol based on the annotation information in the platform.

Identification of differentially expressed genes (DEGs)

The GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r) web tool was used to identify the DEGs between BM tissues of HER2-positive BC and nonmetastatic primary HER2-positive BC samples. Benjamini & Hochberg (false discovery rate) was used to compare differences of genes expression in two groups. $|\log(\text{fold change})| > 1$ and P value <0.01 were considered as threshold parameters.

Gene ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs

DAVID (https://david.ncifcrf.gov/) (version 6.8) is a database for the functional annotation of large lists of genes (22,23). GO analysis is used to identify the molecular

functions (MFs), biological processes (BPs), and cellular components (CCs) associated with the DEGs (24). KEGG database is used for systematic analysis of high-level gene functions and pathways (25). DAVID (version 6.8) from the Bioinformatics website (https://www.bioinformatics.com.cn/) was applied for visualizing the results of the GO and KEGG pathway enrichment analysis. P<0.05 was considered statistically significant.

Protein-protein interaction (PPI) network construction and analysis

STRING (https://string-db.org/) database is used to construct PPI networks (26). In the present study, STRING (version 11.5) database was used to construct a PPI network of DEGs; combined score >0.4 was considered statistically significant. Cytoscape (version 3.7.1) database is used for visualizing the molecular interaction networks (27). MCODE is an application in Cytoscape for identifying densely connected regions in a PPI network based on topology (28). MCODE uses vertex weighting, which is based on the clustering coefficient (Ci). It calculates the neighbor cliquishness Ci = 2n/ki(ki – 1) of a vertex, where n is the number of edges in the neighbor and ki is the vertex size of the neighbor of the vertex. MCODE selection were as follows: MCODE scores >5, degree cut-off =2, node score cut-off =0.2, maximum depth =100, and k-score =2.

Clinical significance analysis

UALCAN database (https://ualcan.path.uab.edu/) was used to perform in-depth analysis of the candidate DEGs by comparing the gene expression levels between 114 normal breast tissues and 1,097 primary BC tissues from breast invasive carcinoma dataset of The Cancer Genome Atlas (TCGA) database (29,30). Furthermore, the gene expression levels between 556 luminal subtype, 37 HER2-positive, 116 TNBC and 114 normal breast tissues from breast invasive carcinoma dataset of TCGA database were compared. The expression levels of candidate genes between 83 HER2-negative and 9 HER2-positive MBC tissues from MBC dataset of TCGA database were also analyzed.

Kaplan-Meier plotter tool (https://kmplot.com/analysis/) was used to assess the survival outcomes of 1,273 HER2-positive BC patients based on the expression levels of the candidate genes. In this study, the association between expression of candidate genes and outcomes of HER2-positive BC patients with or without BM was assessed.

Statistical analysis

Wilcoxon rank sum test was used to compare gene expression differences between groups. Kaplan-Meier survival analysis was used to compare survival differences in two groups. P<0.05 was considered statistically significant.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

Identification of 1,056 DEGs in BM samples of HER2-positive BC

A total of 1,056 DEGs including 767 downregulated and 289 upregulated genes were identified by analyzing the transcriptome data of 19 BM samples of HER2-positive BC and 19 nonmetastatic primary BC samples from the GSE43837 dataset. The heatmap of these DEGs is shown in *Figure 1A*. Principal component analysis (PCA) is used for efficient dimensionality reduction and exploratory visualization of the data from the GSE43837 dataset. The BCBM group and primary BC group displayed different gene patterns (*Figure 1B*).

Functional enrichment analyses of DEGs

Functional enrichment analysis was performed using DAVID to identify biological mechanisms associated with the DEGs. GO analysis results showed that the DEGs were enriched in CC such as cell surface, membrane, extracellular matrix (ECM), focal adhesion, nucleus, and external side of plasma membrane (*Figure 2A*). Furthermore, DEGs were enriched in BP such as ECM organization, cell adhesion, collagen fibril organization, antigen processing and presentation of peptide antigen via major histocompatibility complex (MHC) class I, negative regulation of transforming growth factor beta receptor signaling pathway, and skeletal system development (*Figure 2B*). DEGs were also enriched in MF such as protein binding, metal ion binding, and small ribosomal subunit ribosomal RNA (rRNA) binding (*Figure 2C*). KEGG pathway analysis showed that the DEGs were enriched in pathways associated with autoimmune thyroid disease, dilated cardiomyopathy, graft-versus-host disease (*Figure 2D*).

After removing above one outlier in BCBM group

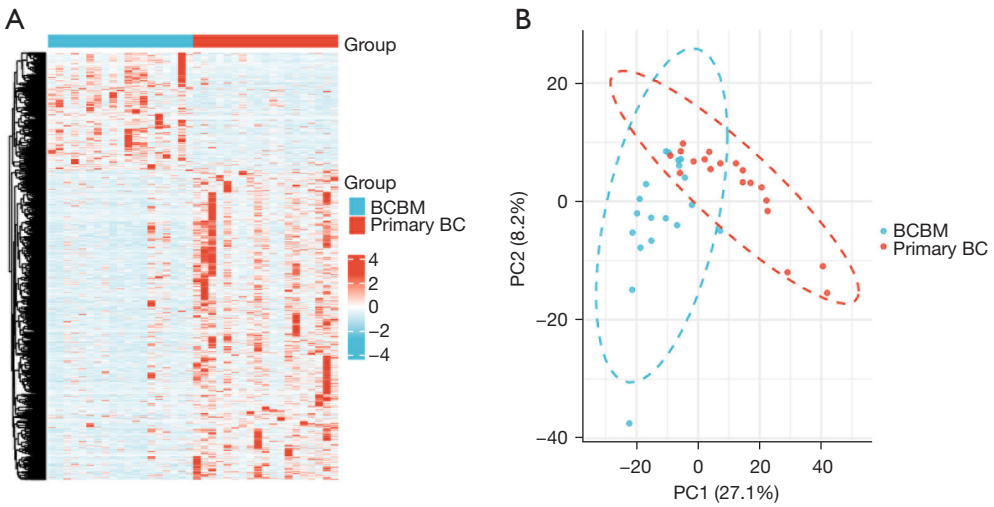

Figure 1 DEGs. (A) Hierarchical clustering of 1,056 DEGs in GES43837. (B) The BCBM group and primary BC group displayed different gene patterns. BCBM, breast cancer with brain metastasis; BC, breast cancer; PC, principal component; DEGs, differentially expressed genes.

and three outliers in primary BC group discovered by PCA analysis, GO analysis and KEGG pathway analysis were performed again. GO analysis results showed that the DEGs were enriched in CC such as ECM, membrane, cytosol, cell surface, and extracellular exosome (Figure S1A). Furthermore, DEGs were enriched in BP such as ECM organization, skeletal system development, positive regulation of protein catabolic process, embryonic limb morphogenesis, and osteoclast differentiation (Figure S1B). DEGs were also enriched in MF such as protein binding, platelet-derived growth factor binding, protein kinase A binding, protease binding, and integrin binding (Figure S1C). KEGG pathway analysis showed that the DEGs were enriched in pathway of neurodegeneration-multiple diseases, amoebiasis, rheumatoid arthritis, dilated cardiomyopathy, and advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling pathway in diabetic complications (Figure S1D).

PPI network and module construction to identify hub genes

PPI network analysis of the DEGs was performed using the STRING 11.5 database (*Figure 3A*). Fourteen DEGS in the most significant module as visualized using the MCODE plugin of Cytoscape 3.7.1 (*Figure 3B*). Among these 14 DEGs, SOX9 was upregulated and the remaining 13 DEGs (*FN1*, *COL1A2*, *COL3A1*, *MMP3*, *MMP13*, *MMP14*, *POSTN*, *VCAN*, *TGFB1*, *LUM*, *CD44*, *ITGB1*, and *ITGB2*) were downregulated.

After removing above outliers, PPI network analysis was also performed (Figure S2A). Thirteen DEGs were identified by the MCODE plugin of Cytoscape 3.7.1 (Figure S2B). Among these 13 DEGs, SOX9 and ACTB were upregulated and the remaining 11 DEGs (*COL1A1*, *COL1A2*, *COL3A1*, *MMP3*, *MMP13*, *MMP14*, *POSTN*, *VCAN*, *TGFB2*, *TGFB1*, *LUM*, and *ITGB1*) were downregulated.

Clinical significance analysis

UALCAN was used to compare the expression levels of the 14 DEGs in clinical BC samples. Firstly, the expression levels of the 14 DEGs between 114 normal breast tissues and 1,097 primary BC tissues from breast invasive carcinoma dataset of TCGA database was compared. The results showed upregulation of *FN1*, *COL1A2*, *COL3A1*, *MMP3*, *MMP13*, *MMP14*, *POSTN*, *VCAN*, *TGFB1*, and *LUM*, and downregulation of *CD44*, *SOX9*, and *ITGB1* in the primary BC samples compared to the normal breast tissue samples (*Figure 4*). The differences in the expression levels of the 14 DEGs between normal breast tissues and different BC subtypes were then analyzed. The results showed upregulation of *FN1*, *COL1A2*, *COL3A1*, *MMP3*, *MMP13*, *MMP14*, *POSTN*, *VCAN*, and *TGFB1* in all the subtypes of BC, and upregulation of LUM in the luminal and HER2-positive BC tissue samples (*Figure 5*).

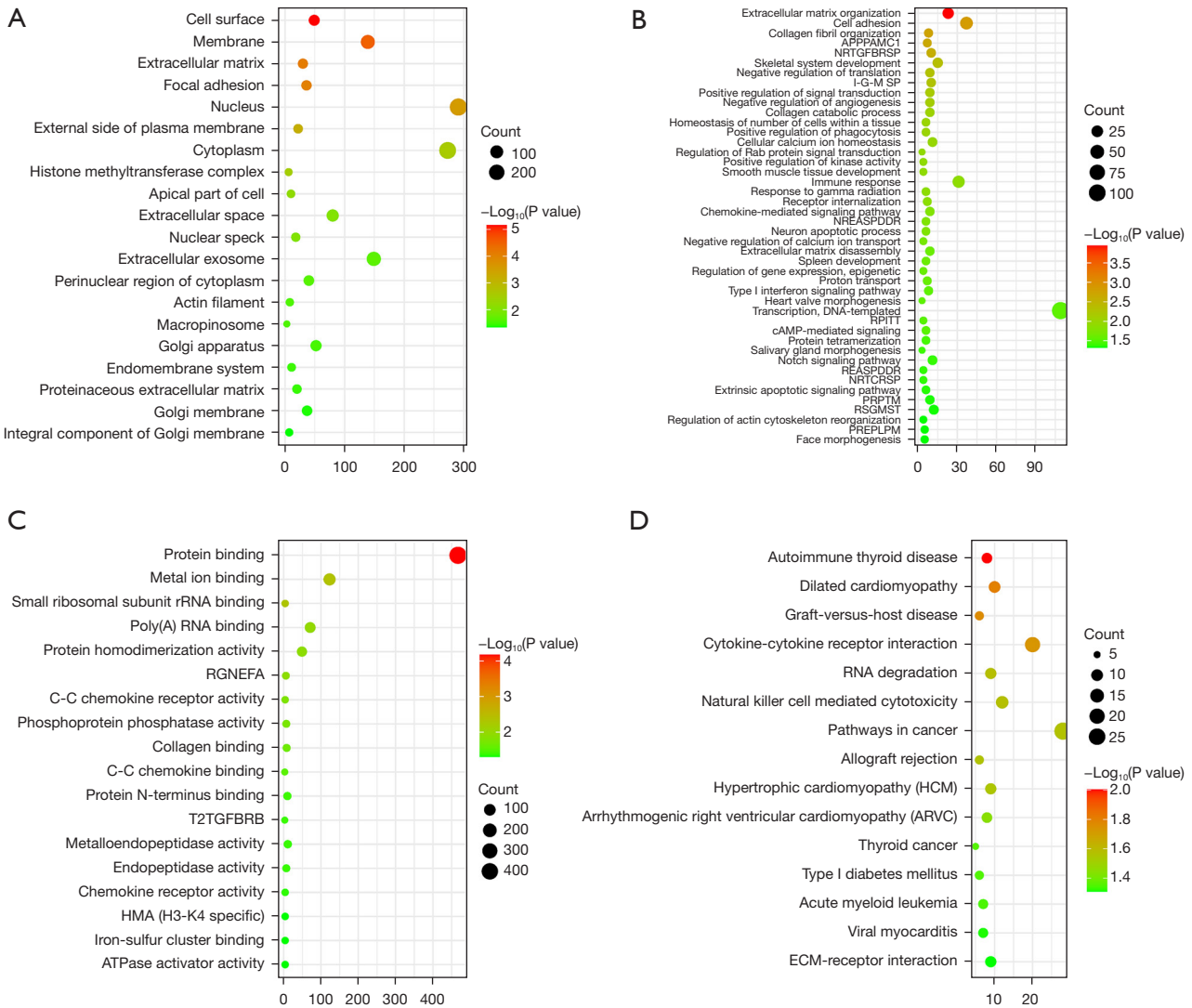

Figure 2 GO and KEGG pathway enrichment analysis of DEGs in BCBM. (A) GO CC of DEGs. (B) GO BP of DEGs. (C) GO MF of DEGs. (D) KEGG of DEGs. APPPAMC1, antigen processing and presentation of peptide antigen via MHC class I; NRTGFBRSP, negative regulation of transforming growth factor beta receptor signaling pathway; I-G-M SP, interferon-gamma-mediated signaling pathway; NREASPDDR, negative regulation of extrinsic apoptotic signaling pathway via death domain receptors; RPITT, regulation of potassium ion transmembrane transport; REASPDDR, regulation of extrinsic apoptotic signaling pathway via death domain receptors; NRTCRSP, negative regulation of T cell receptor signaling pathway; PRPTM, positive regulation of protein targeting to mitochondrion; RSGMST, regulation of small GTPase mediated signal transduction; PREPLPM, positive regulation of establishment of protein localization to plasma membrane; rRNA, ribosomal RNA; RGNEFA, Rab guanyl-nucleotide exchange factor activity; T2TGFBRB, type II transforming growth factor beta receptor binding; HMA, histone methyltransferase activity; ECM; extracellular matrix; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BCBM, breast cancer with brain metastasis; CC, cellular component; BP, biological process; MF, molecular function.

Furthermore, SOX9 and ITGB1 were downregulated in all subtypes of BC, whereas CD44 was downregulated in the luminal and TNBC tissue samples (*Figure 5*). Finally, the differences in the gene expression levels of the 14 DEGs between 83 HER2-negative MBC and 9 HER2-positive MBC tissue samples was compared. The results showed

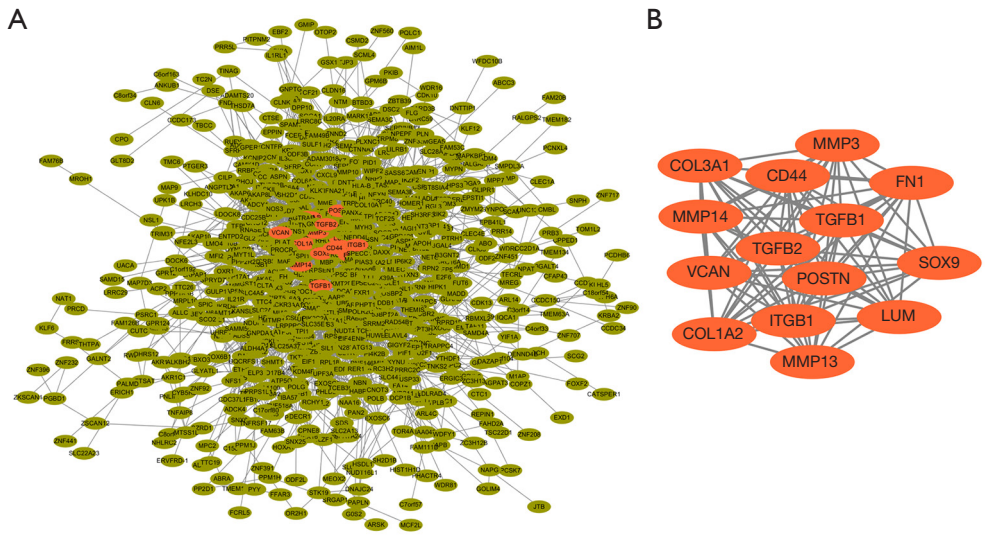

Figure 3 PPI network and the most significant module of DEGs. (A) PPI network of DEGs. (B) Most significant modules obtained from PPI network. PPI, protein-protein interaction; DEGs, differentially expressed genes.

higher expression of *FN1*, *COL1A2*, *COL3A1*, and *POSTN* and reduced expression of *CD44* in the HER2-positive MBC tissue samples compared to the HER2-negative MBC tissue samples (*Figure 6*). The other 9 candidate genes had no differences between HER2-positive MBC and HER2-negative MBC tissue samples in current study (Figure S3).

Kaplan-Meier survival curve analysis was performed to determine the differences in the overall survival (OS) and relapse-free survival (RFS) of the 1,273 HER2-positive BC patients based on the expression levels of the 14 candidate genes. HER2-positive patients with lower expression of *CD44* showed significantly lower OS (P=0.011, *Figure 7A*) than those with high *CD44* expression. Furthermore, OS rates were significantly lower for the HER2-positive BC patients with higher expression levels of *COL1A2* (P=0.098, *Figure 7B*), *MMP14* (P=0.52, *Figure 7C*), *POSTN* (P=0.14, *Figure 7D*), and *SOX9* (P=0.21, *Figure 7E*) compared to those with lower expression of the corresponding genes. HER2-positive patients with lower expression of *CD44* also showed significantly lower RFS (P=0.00061, *Figure 8A*) than those with high *CD44* expression. Furthermore, RFS rates were also significantly lower for the HER2-positive BC patients with higher expression levels of *COL1A2* (P=0.024, *Figure 8B*), *MMP14* (P=0.021, *Figure 8C*), *POSTN* (P=0.006, *Figure 8D*), and *SOX9* (P=0.0093, *Figure 8E*) compared to those with lower expression of the corresponding genes. The survival outcomes of the HER2-positive patients did not show any statistically significant differences based on the differential expression levels of the remaining 9 candidate genes.

Discussion

Nearly 30–50% of patients with MBC develop BM (2,3). Furthermore, the risk of BM is higher in patients with HER2-positive/hormone receptor (HR)-negative and TNBC (3). The mortality rate is 50% for HER2-positive BC patients with BM progression (18). In previous studies, researchers fully elucidated the gene expression patterns of BCBM (31,32). However, their results are not independent of HER2 status which will affect the gene expression profiles and management strategies. Although Kuroiwa *et al.* (33) has explored the expression signature of BM in HER2-positive BC, the screening processes are based on cell line and mouse xenograft which are different from the microenvironment of human BCBM. One study based on bioinformatic analysis has identified some hub genes in BM of HER2 positive BC (34). However, the genes they screened are not verified by data from other databases, to some extent leading to unreliability of their clinical application.

In present study, microarray analysis of a GEO BC dataset identified 1,056 DEGs, including 767 downregulated genes and 289 upregulated genes between BM tissues of HER2-positive BC and primary BC tissues. Functional enrichment analysis of the DEGs showed

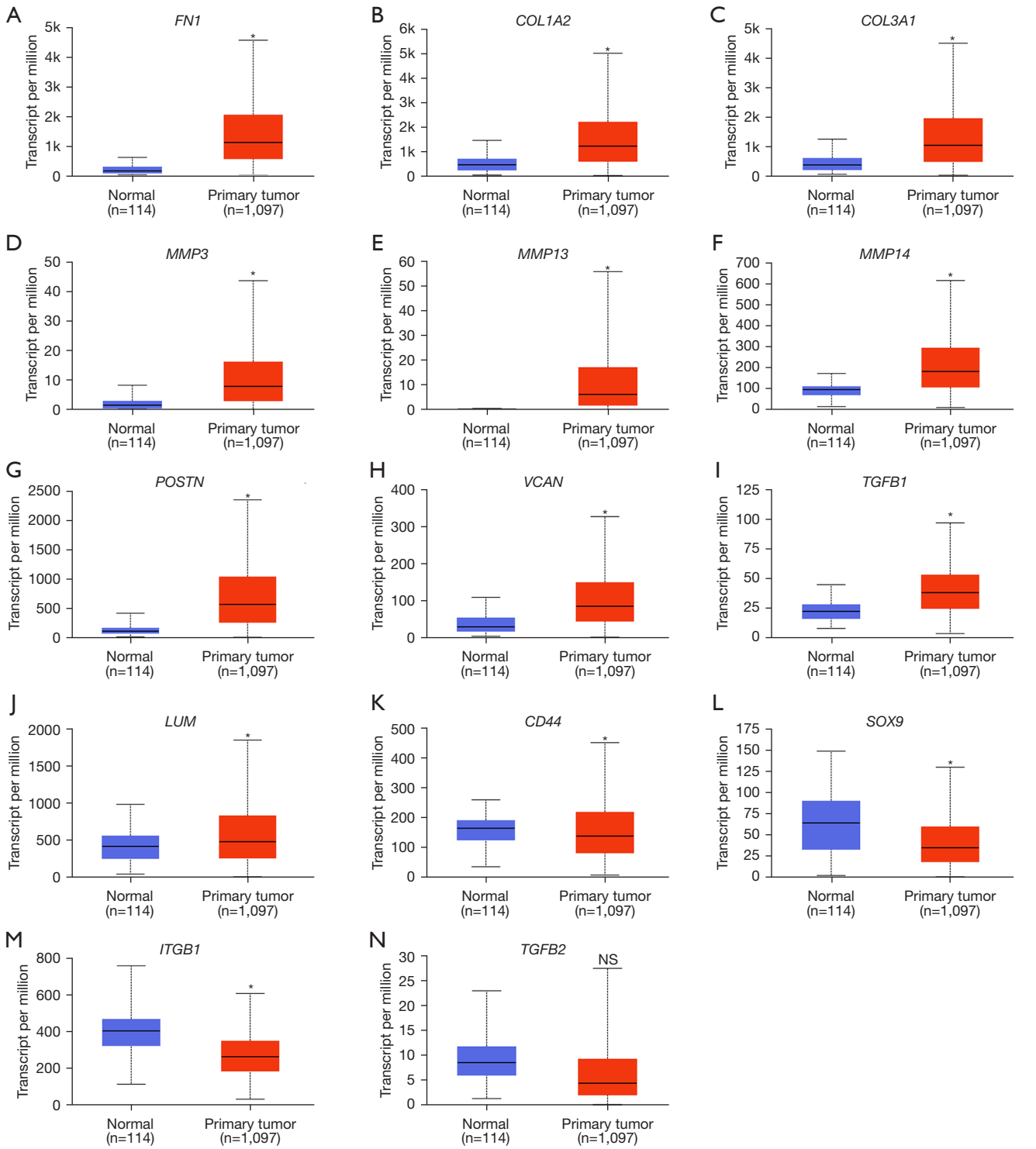

Figure 4 Comparation of 14 hub genes expression between primary BC and normal tissues via UALCAN. (A) *FN1* expression level. (B) *COL1A2* expression level. (C) *COL3A1* expression level. (D) *MMP3* expression level. (E) *MMP13* expression level. (F) *MMP14* expression level. (G) *POSTN* expression level. (H) *VCAN* expression level. (I) *TGFB1* expression level. (J) *LUM* expression level. (K) *CD44* expression level. (L) *SOX9* expression level. (M) *ITGB1* expression level. (N) *TGFB2* expression level. *, P<0.05. NS, no significance; BC, breast cancer.

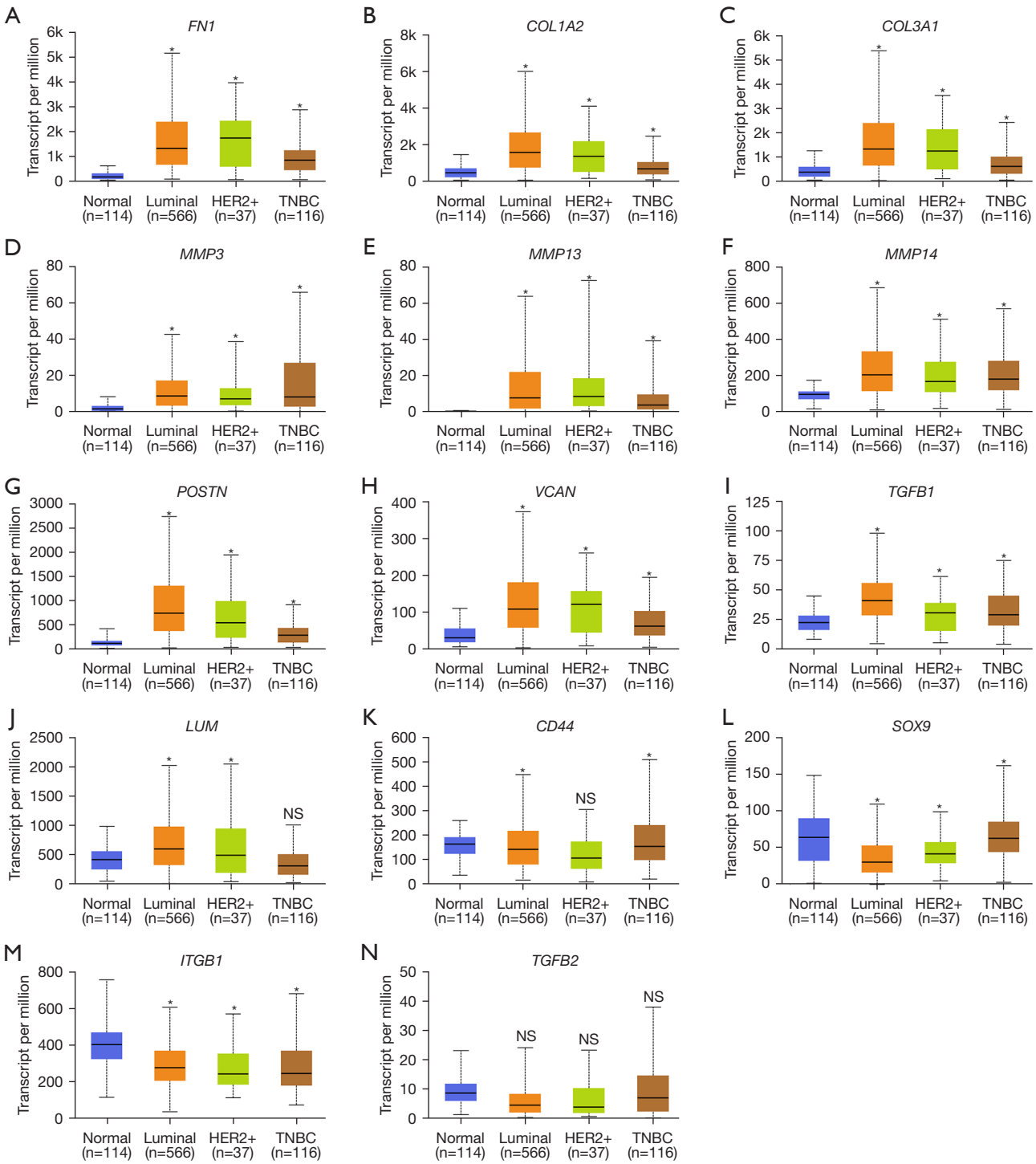

Figure 5 Comparation of 14 hub genes expression between different subtypes of BC and normal tissues via UALCAN. (A) *FN1* expression level. (B) *COL1A2* expression level. (C) *COL3A1* expression level. (D) *MMP3* expression level. (E) *MMP13* expression level. (F) *MMP14* expression level. (G) *POSTN* expression level. (H) *VCAN* expression level. (I) *TGFB1* expression level. (J) *LUM* expression level. (K) *CD44* expression level. (L) *SOX9* expression level. (M) *ITGB1* expression level. (N) *TGFB2* expression level. *, P<0.05; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; NS, no significance; BC, breast cancer.

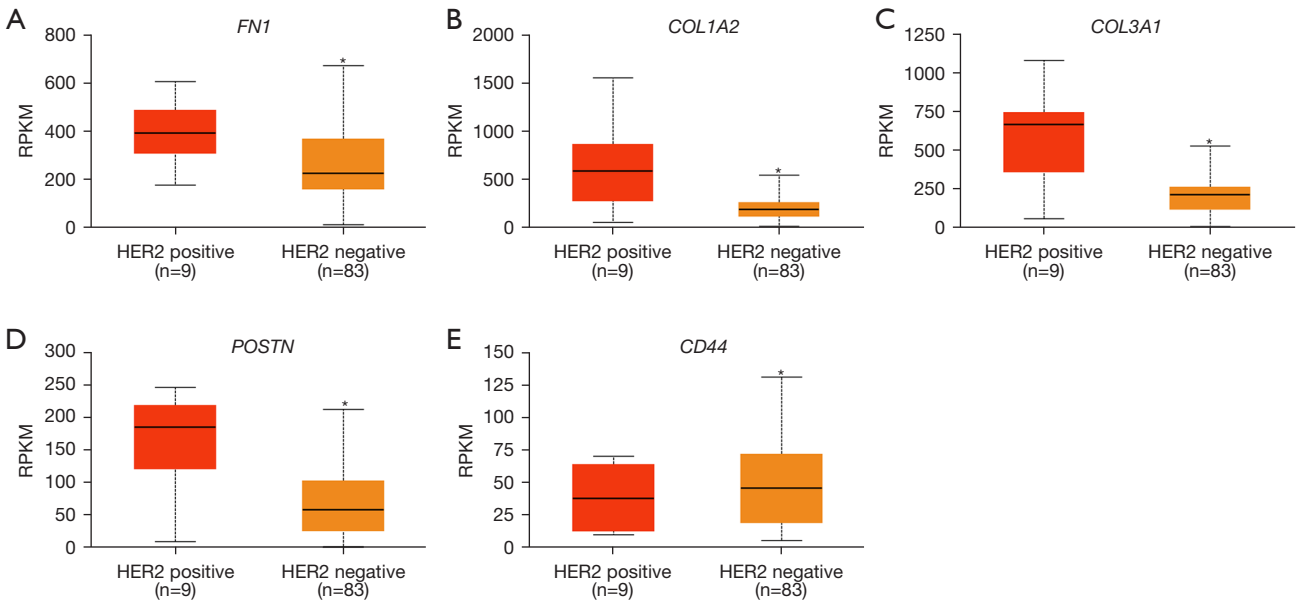

Figure 6 Different expressed hub genes expression between HER2-positive and negative MBC via UALCAN. (A) *FN1* expression level. (B) *COL1A2* expression level. (C) *COL3A1* expression level. (D) *POSTN* expression level. (E) *CD44* expression level. *, P<0.05. RPKM, reads per kilobase per million mapped reads; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer.

enrichment of pathways related to ECM organization, cell adhesion, and collagen fibril organization. Furthermore, 14 hub genes were identified as candidate genes regulating BM by the hierarchical clustering and PPI network analysis of the DEGs. Among these, *SOX9* was upregulated and the remaining 13 DEGs were downregulated. PCA plot was also performed, and the BCBM group and primary BC group displayed different gene patterns. After removed one outliner in BCBM group and three outliers in primary BC group, GO analysis and KEGG pathway analysis was performed, and PPI network was constructed again. As functional enrichment is largely consistent, and most of the hub genes are overlapped, outlier samples were considered to be caused by tumor heterogeneity.

CD44 is an integral membrane protein that plays a pivotal role in cellular signaling and cell-cell communication, and also acts as a link between the ECM components and the intracellular cytoskeletal proteins (35,36). CD44 is a commonly used cancer stem cell (CSC) marker. *CD44* overexpression is associated with cancer cell proliferation, metastasis, invasion, migration and stemness, and tumor resistance to chemotherapy and/or radiotherapy in several cancers (37). The relationship between *CD44* expression levels and the clinicopathological features and survival outcomes of BC patients is not clear. In the present study, *CD44* expression was significantly lower in the HER2-positive BCBM patients compared to the primary HER2-positive BC patients. Furthermore, *CD44* was downregulated in BC, especially in the luminal BC and TNBC. Moreover, *CD44* expression was significantly lower in the HER2-positive MBC compared to the HER2-negative MBC. OS and RFS rates were significantly worse for HER2-positive BC patients with low *CD44* expression compared to those with higher *CD44* expression.

The metastatic cascade involves local invasion through the basement membrane and the surrounding ECM, intravasation into the vessel or the lymphatic vessels, and subsequent dissemination to the distant sites (38). Epithelial-mesenchymal transition (EMT) and ECM remodeling are required for the initiation of cancer metastasis (39). Collagen is a major component of the ECM that is also involved in the development of human placenta (40). Matrix metalloproteinases (MMPs) are a family of calcium and zinc dependent proteases that play a key role in degrading the ECM proteins (41). *MMP13* was first identified in BC as a key player in the activation cascade of the extracellular MMPs and ECM degradation (42). *MMP13* promotes the initiation, growth, migration, and invasion of BC cells and is associated with aggressive BC phenotypes and poorer survival outcomes (43,44). In the

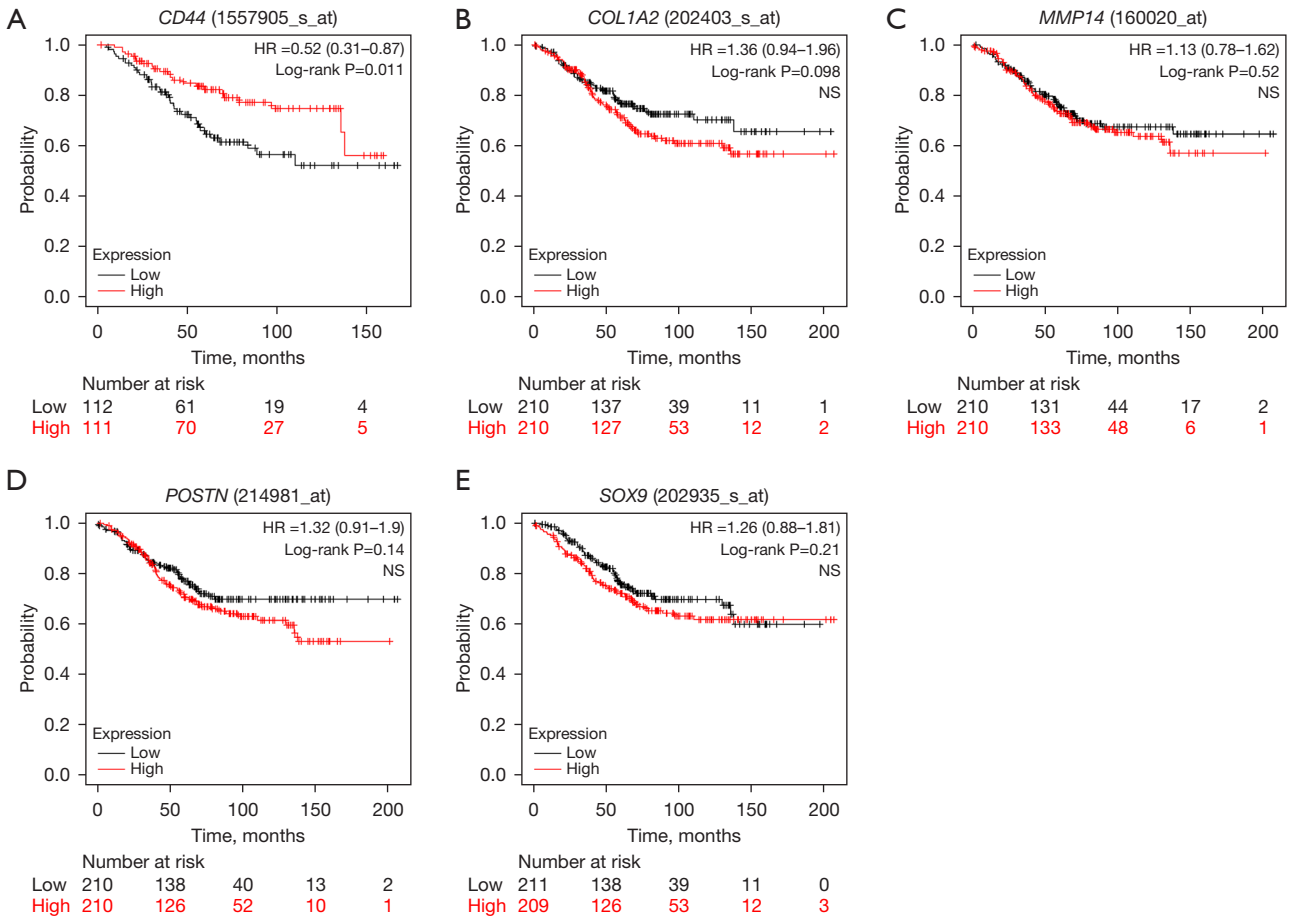

Figure 7 OS of hub genes in HER2-positive BC via Kaplan-Meier plotter. (A) OS of *CD44* in HER2-positive BC. (B) OS of *COL1A2* in HER2-positive BC. (C) OS of *MMP14* in HER2-positive BC. (D) OS of *POSTN* in HER2-positive BC. (E) OS of *SOX9* in HER2-positive BC. HR, hazard ratio; NS, no significance; OS, overall survival; HER2, human epidermal growth factor receptor 2; BC, breast cancer.

animal models, *MMP13* promoted lung metastasis and played a key role in the differentiation and activation of osteoclasts during BM (45,46). *MMP3* is another MMP that is downregulated in BCBM compared to the primary BC tissues (47). However, *MMP3* activity was higher in metastatic brain tissues compared to the normal brain tissues (48). The expression levels of *MMP3* and *MMP14* in brain-derived clones of MDA-MB-231 did not show significant differences compared to the normal brain tissues (49). *MMP14* (also known as membrane type 1-MMP) is an activator of *MMP2* and promotes cancer cell invasion, metastasis and angiogenesis by degrading ECM and cell adhesion proteins (50,51). *MMP14* is downregulated in BCBM and associated with metastasis and poorer survival outcomes (52). In the present study, *MMP3*, *MMP13*, and *MMP14* were upregulated in all the subtypes of BC, and their expression levels were independent of the HER2 status in MBC. HER2-positive BC patients with high *MMP14* expression were associated with poorer RFS.

COL1A2 and *COL3A1* encode the α2 chain of type I collagen and the α1 chain of types I and III collagens, respectively; moreover, type I and III collagens are important components of the ECM (53,54). Type I and III collagens participate in tumor invasion and progression (54-56). Furthermore, aberrant expression of *COL1A2* is associated with survival outcomes in several cancers (57-61). The upregulation of *COL1A2* and *COL3A1* is associated with poor survival outcomes in patients with ER-positive BC (62,63). Furthermore, the expression levels of *COL1A2* and *COL3A1* are upregulated in BC after radiotherapy (64). A previous study showed that low expression of *COL3A1* correlated with metastasis in patients with BCBM and poor

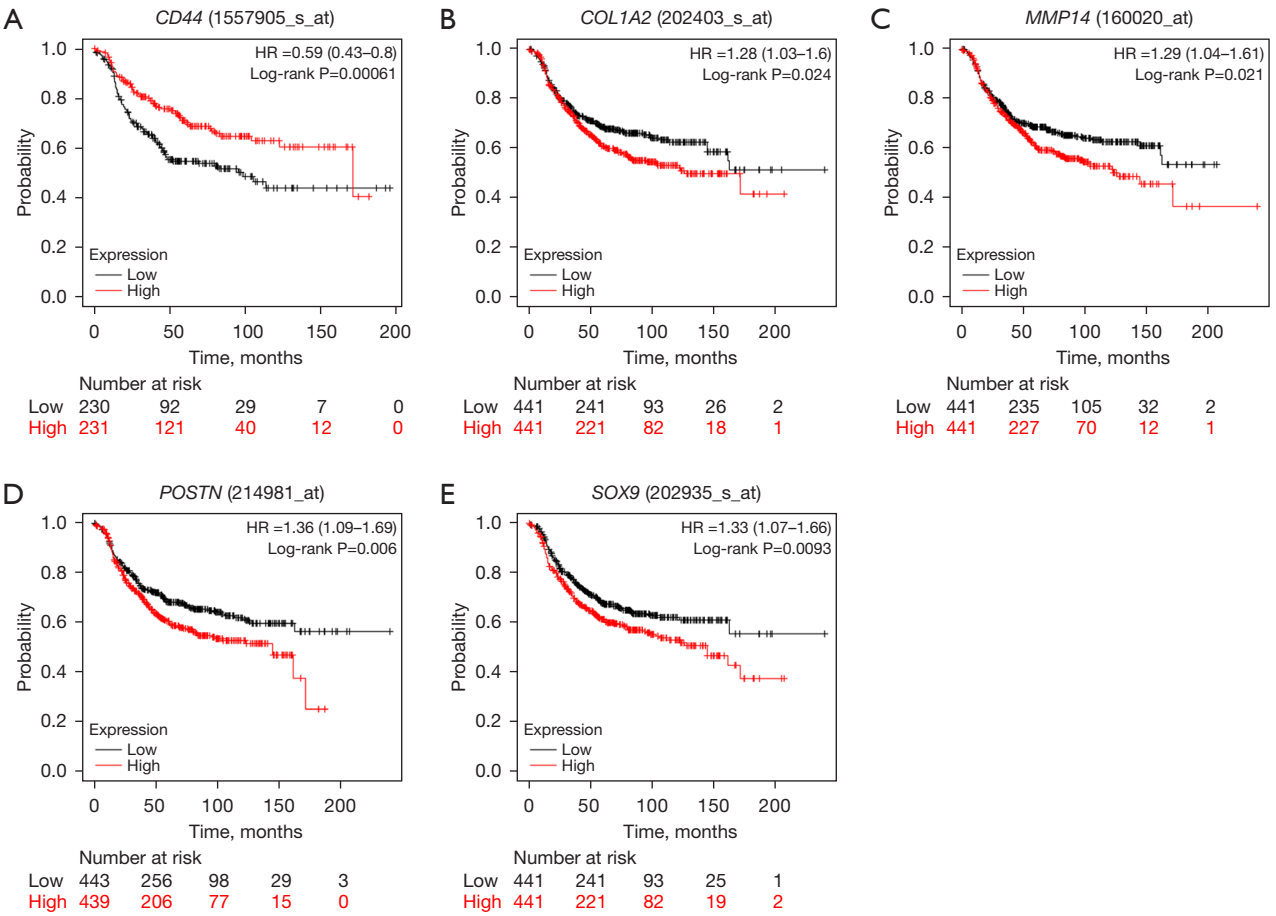

Figure 8 RFS of hub genes in HER2-positive BC via Kaplan-Meier plotter. (A) RFS of *CD44* in HER2-positive BC. (B) RFS of *COL1A2* in HER2-positive BC. (C) RFS of *MMP14* in HER2-positive BC. (D) RFS of *POSTN* in HER2-positive BC. (E) RFS of *SOX9* in HER2-positive BC. HR, hazard ratio; RFS, relapse-free survival; HER2, human epidermal growth factor receptor 2; BC, breast cancer.

survival outcomes (52). In this current study, *COL1A2* and *COL3A1* were downregulated in HER2-positive BCBM patients. However, further investigation showed that *COL1A2* and *COL3A1* were upregulated in all the subtypes of BC. Among MBC patients, HER2-positive BC group showed higher expression levels of *COL1A2* and *COL3A1*. Furthermore, HER2-positive BC patients with higher *COL1A2* expression showed poor RFS and OS compared to those with lower *COL1A2* expression.

ECM proteins and secretory factors mediate tumor-stromal interactions (65). *POSTN*, also known as *OSF-2*, is a secretory cell-adhesion glycoprotein in the ECM that plays a critical role in tumor cell proliferation, adhesion, migration, and EMT (66). *POSTN* is also implicated in cancer cell stemness and regulates tumor angiogenesis, lymph-angiogenesis, and distant metastases (67,68). *POSTN* is highly expressed in the tumor stromal cells such as the cancer-associated fibroblasts in BC, and its upregulation correlates with tumor malignancy and shorter survival rates of the IDC patients (68-70). A positive feedback loop between *POSTN* and *TGF-β* promotes and maintains the stemness of cancer cells, and is associated with increased invasion and worse survival outcomes (71-73). In the GEO-BC dataset, *POSTN* and *TGFB2* were downregulated in the HER2-positive BM. However, in further studies, *POSTN* was upregulated in all the subtypes of BC.

SOX9 is a member of the SOX family of transcription factors and is associated with tumorigenesis and poor survival outcomes in solid tumors (74). In non-small cell lung cancer (NSCLC), tumor-associated macrophages (TAMs) secrete *TGF-β*, which induces *SOX9* expression via the c-Jun/SMAD3 pathway and subsequently promotes

tumor progression and metastasis (75). *SOX9* promotes BC growth, proliferation, migration, invasion, and metastasis by directly regulating genes involved in cellular apoptosis and EMT (76-78). Sox9 upregulation is associated with the $CD44^{+}/CD24^{-/low}$ phenotype and poor prognosis in BC (79). The upregulation, deacetylation, and nuclear localization of *SOX9* is associated with tamoxifen resistance in BC (80). In the GEO dataset, *SOX9* was upregulated in BM samples of HER2-positive BC. However, *SOX9* was downregulated in all the subtypes of BC, and its expression was independent of the HER2 status. Furthermore, HER2-positive BC patients with higher *SOX9* expression were associated with poor RFS and OS than those with low *SOX9* expression.

FN1 promotes EMT and cancer cell migration (81,82). *LUM* inhibits MMPs and BC progression, and is co-expressed with COL1A2 in BCBM (83). *VCAN* is associated with poor OS in BC (84-86). This study showed that the expression levels of *FN1*, *LUM*, and *VCAN* were significantly lower in BM samples of HER2-positive BC than the primary BC patients. However, further verification demonstrated upregulation of *FN1*, *LUM*, and *VCAN* in primary BC tissues. Furthermore, *FN1* was highly expressed in HER2-positive MBC compared to the HER2-negative MBC patients. *ITGB1*, the β1 integrin subunit, mediates initiation, growth, and progression of BC (87). In the present study, *ITGB1* was downregulated in primary BC independent of the subtypes and in the HER2-positive BCBM patients.

Several downregulated genes in HER2-positive BCBM patients were upregulated in majority of the BC subtypes and were associated with poorer survival outcomes. This suggested that the tumor microenvironment was different in patients with BM compared to those without BM. Previous studies showed that *COL1A2*, *COL3A1*, *MMP3*, *MMP14*, *MMP2*, and *FN* were downregulated in MBC samples compared to the primary BC samples. These genes are potentially involved in the interactions of the MBC cells with the microenvironment and may be necessary for the metastasis to the lymph nodes and their survival in the new microenvironment (88-91). Another hypothesis is that some of these genes are mainly expressed in the stromal tissues. However, stromal tissue levels are significantly lower in the brain compared to the primary BC. Furthermore, patients with *LUM*-positive cancer cells were associated with longer survival rates, but patients with *LUM*-positive stromal tissues were associated with shorter survival rates (92). This suggested different biological roles for some EMT and ECM genes in the stroma and the cancer cells, especially in the context of BM.

Conclusions

In conclusion, 1,056 DEGs (767 downregulated and 289 upregulated) and 14 hub genes were identified in BM samples of HER2-positive BC. Furthermore, *CD44*, *COL1A2*, *MMP14*, *POSTN*, and *SOX9* were associated with BM in BC and survival outcomes of patients with HER2-positive BC. These genes played a significant role in the metastatic tumor microenvironment. However, as present results are based on a single database, bias is existed in current investigation. In addition, above hub genes are components of the stromal compartment which are required for normal tissue homeostasis. Although it has been known that the homeostasis is re-established during BM, it is still difficult to find proper way to keep balance between BM management and tissue homeostasis when targeting the hub genes. Further studies are still required to decipher the biological roles and mechanisms of these hub genes in BM of HER2-positive BC patients.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2715/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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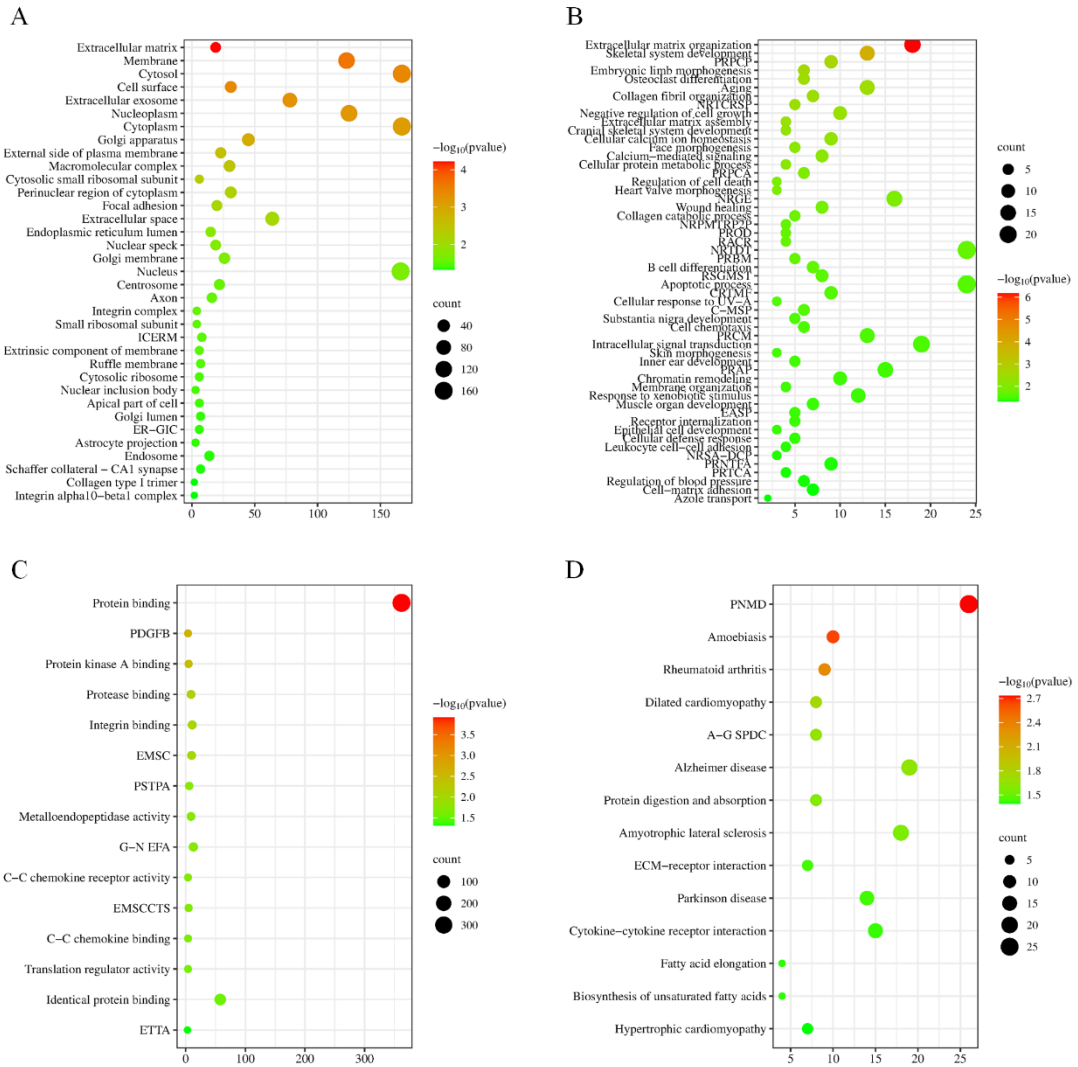

Figure S1 GO and KEGG pathway enrichment analysis of DEGs in BCBM after removing the outliers. (A) GO CC of DEGs. (B) GO BP of DEGs. (C) GO MF of DEGs. (D) KEGG of DEGs. ICERM, integral component of endoplasmic reticulum membrane; ER-GIC, endoplasmic reticulum-Golgi intermediate compartment; PRPCP, positive regulation of protein catabolic process; NRTCRSP, negative regulation of T cell receptor signaling pathway; PRPCA, positive regulation of protein complex assembly; NRGE, negative regulation of gene expression; NRPMTRP2P, negative regulation of pri-miRNA transcription from RNA polymerase II promoter; miRNA, microRNA; PROD, positive regulation of osteoclast differentiation; RACR, regulation of actin cytoskeleton reorganization; NRTDT, negative regulation of transcription; DNA-templated; PRBM, positive regulation of bone mineralization; RSGMST, regulation of small GTPase mediated signal transduction; CRTMF, cellular response to tumor necrosis factor; C-MSP, chemokine-mediated signaling pathway; PRCM, positive regulation of cell migration; PRAP, positive regulation of apoptotic process; EASP, extrinsic apoptotic signaling pathway; NRSA-DCP, negative regulation of substrate adhesion-dependent cell spreading; PRNTFA, positive regulation of NF-kappaB transcription factor activity; PRTCA, positive regulation of T cell activation; PDGFB, platelet-derived growth factor binding; EMSC, extracellular matrix structural constituent; PSTPA, protein serine/threonine phosphatase activity; G-N EFA, guanyl-nucleotide exchange factor activity; EMSCCTS, extracellular matrix structural constituent conferring tensile strength; ETTA, efflux transmembrane transporter activity; PNMD, pathways of neurodegeneration-multiple diseases; A-G SPDC, AGE-RAGE signaling pathway in diabetic complications; AGE, advanced glycation end product; RAGE, receptor for AGE; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BCBM, breast cancer with brain metastases; CC, cellular component; BP, biological process; MF, molecular function.

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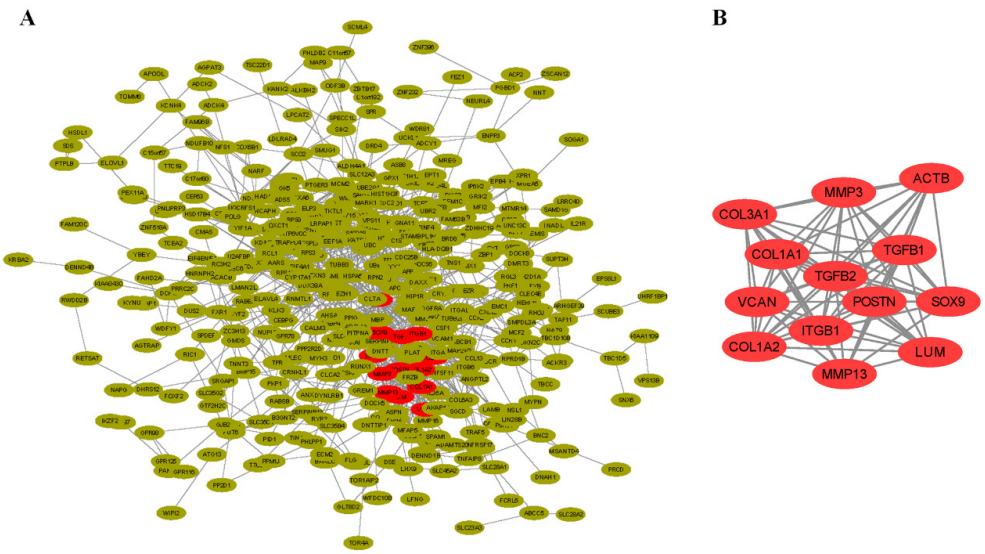

Figure S2 PPI network and the most significant module of DEGs after removing the outliers. (A) PPI network of DEGs. (B) Most significant modules obtained from PPI network. PPI, protein-protein interaction; DEGs, differentially expressed genes.

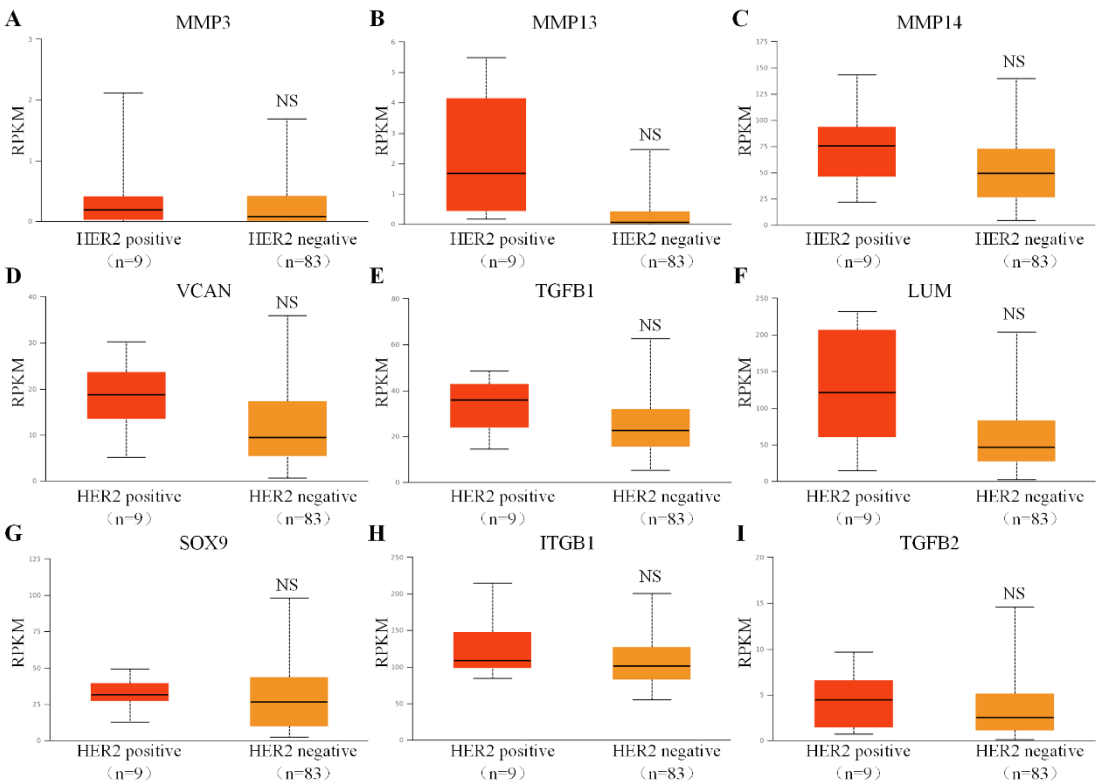

Figure S3 Comparation of 9 candidate hub genes expression between HER2-positive and negative MBC via UALCAN. (A) *MMP3* expression level. (B) *MMP13* expression level. (C) *MMP14* expression level. (D) *VCAN* expression level. (E) *TGFB1* expression level. (F) *LUM* expression level. (G) *SOX9* expression level. (H) *ITGB1* expression level. (I) *TGFB2* expression level. RPKM, reads per kilobase per million mapped reads; HER2, human epidermal growth factor receptor 2; NS, no significance; MBC, metastatic breast cancer.

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