

Comprehensive Analysis of the Expression, Prognosis, and Biological Significance of OVOLs in Breast Cancer

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Background: The study aimed to investigate the expression of OVOLs in breast cancer (BRCA) tissues and their value in prognosis.

Methods: ONCOMINE was used to analyze the expressions of OVOL1, OVOL2, and OVOL3 mRNA between BRCA tissues and normal breast tissues. The Wilcoxon rank sum test and *t*-test were used to assess the expression of OVOLs between BRCA tissues and unpaired/paired normal breast tissues. GEPIA and ROC curves were used to analyze the relationship between OVOLs expression and clinical pathological stage. Kaplan–Meier plotter was used to analyze prognosis. cBioPortal was used to analyze the mutation of OVOLs. GEPIA was used to analyze the co-expression of OVOLs. GO and KEGG analyses were performed by the DAVID software to predict the function of OVOLs co-expression genes.

Results: The expression of OVOL1/2 was significantly higher in BRCA tissues than in normal breast tissues. The OVOL3 expression correlated with tumor stage. The AUC of OVOLs was 0.757, 0.754, and 0.537, respectively. OVOL1 high expression was associated with shorter overall survival (HR: 1.48; 95% CI: 1.07–2.04; P=0.018). The OVOLs were associated with pathways including axon guidance, thyroid hormone signaling pathway, and ubiquinone and other terpenoid-quinone biosynthesis.

Conclusion: OVOL1 is a new potential marker of prognosis in BRCA, and OVOL1/2 are potential therapeutic targets in BRCA.

Keywords: OVOLs, breast cancer, prognostic, bioinformatics, gene expression

Introduction

Breast cancer (BRCA) is the most common malignancy in women and the leading cause of cancer deaths in women worldwide.¹ Despite the prognostic benefits of local surgery, conventional chemotherapy, precision radiotherapy, endocrine therapy, and the use of monoclonal antibodies for BRCA patients.^{2,3} Prognosis of BRCA is predicted in relation to clinical, pathological, and molecular features.⁴ Molecular biomarkers have been identified as promising candidates not only to predict biological behavior and clinical outcomes, but also to help improve therapeutic options.^{5,6} Therefore, the identification of new prognostic markers could provide new insights into the early detection of BRCA and reduce mortality and recurrence.

Currently, there are no validated screening tests for early BRCA detection, and there is a need to explore genetic signatures associated with prognostic prediction of

the underlying mechanisms of BRCA progression. Therefore, studying the prognostic value of OVOLs for patients with BRCA may help to improve the prediction of clinical prognosis in BRCA and inform personalized treatment.

OVOLs (OVO-like proteins) are universally conserved genes encoding C2H2 zinc finger transcription factors in mammals.⁷ OVOLs act as transcription factors to regulate gene expression in various differentiation processes.⁸ OVOLs contains three members, including OVOL1, OVOL2, and OVOL3. Epithelial-to-mesenchymal transition (EMT) plays a key role in the stromal invasion of tumor cells.⁹ Multiple signaling pathways and various molecules are involved in this process; OVOL1 and OVOL2, two transcription factors, have been identified as key guardians that inhibit EMT and facilitate its mirror process, mesenchymal to epithelial conversion.^{10,11}

In recent years, OVOLs are associated with clinical staging, EMT, tumor metastasis, can modulate cancer cell stemness, and are promising markers for prognostic prediction.^{11–17} However, the roles of OVOLs in BRCA are unclear. In this study, bioinformatics was used to analyze the potential of OVOLs as a predictor of breast carcinogenesis and their regulatory network.

Materials and Methods

Differential Expression of OVOLs

Oncomine (<https://www.oncomine.org/resource/login.html>) were used to analyze the levels of OVOLs mRNAs in BRCA tissues and normal tissues.¹⁸ The screening criteria were $P < 0.05$, fold change > 1.5 , and Top 10% of gene rank.

Software: R (version 3.6.3) (statistical analysis and visualization). R package: mainly ggplot2 (for visualization). Molecules: OVOL1 [ENSG00000172818], OVOL2 [ENSG00000125850], and OVOL3 [ENSG00000105261]. Data: RNAseq data in level 3 HTSeq-FPKM format from the TCGA (<https://portal.gdc.cancer.gov/>) BRCA (breast invasive carcinoma) project. Extracted BRCA of TCGA and corresponding normal tissue data in GTEx. Data filtering: None or retain paired samples. Data conversion: RNAseq data in FPKM (Fragments Per Kilobase per Million) format converted to TPM (transcripts per million reads) format and log₂ transformed. Significance markers: ns, $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The Relationship Between OVOLs and Clinical Characteristics of BRCA

GEPIA (<http://gepia.cancer-pku.cn/>) was used to do OVOLs expression analysis.¹⁹ The expression of OVOLs at different clinical stages was generated online. The co-expression genes of OVOLs were generated online.

ROC curves. Software: R (version 3.6.3). R packages: mainly the pROC package (for analysis) || ggplot2 package. Molecule: OVOL1/2/3. Clinical variables: Tumor vs Normal. Data: UCSC XENA RNAseq data in TPM format for TCGA and GTEx processed uniformly by the Toil process.²⁰ Extracted BRCA for TCGA and corresponding normal tissue data in GTEx. Data filtering: None. Data transformation: RNAseq data in TPM format and log₂ transformed for analysis.

The Relationship Between OVOLs and Prognosis of BRCA

Kaplan–Meier Method. Software: R (version 3.6.3). R package: survminer package (for visualization), survival package (for statistical analysis of survival data). Molecule: OVOL1/2/3. Subgroups: 0–50 vs 50–100. Prognosis type: Overall Survival, Disease specific Survival, and Progression-free Survival. Data: RNAseq data and clinical data in level 3 HTSeq-FPKM format from the TCGA BRCA project. Data filtering: Retain data with clinical information. Data conversion: RNAseq data in FPKM format were converted to TPM format and analyzed by grouping them according to molecular expression. Additional data: prognostic data from an article.²¹

The Mutation and Co-Expression of OVOLs in BRCA

cBioPortal (http://www.cbioportal.org/index.do?session_id=5b4c1773498eb8b3d566f7b8) was used to analyze the mutation of OVOLs in BRCA.

GO and KEGG Analysis

DAVID database was used to do Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for the co-expression genes of OVOLs, including BP (biological process), MF (molecular function), CC (cellular component), and pathway analysis.²²

Statistical Analysis

All statistical analyses were performed using R (v.3.6.3). The expression of OVOLs between BRCA tissue and

normal breast tissue was analyzed using the Wilcoxon rank sum test. The expression of OVOLs between BRCA tissues and paired normal breast tissues was analyzed using *t*-test. P values less than 0.05 were considered statistically significant.

Results

OVOLs Expression in BRCA and Normal Breast Tissues

As shown in [Figure 1](#) and [Table 1](#), OVOL1/2 mRNA expression in BRCA tissues was significantly higher than that in normal breast tissues ($P<0.05$). OVOL2 had the highest expression change (fold change=3.638, $P<0.05$), and 1 data set confirmed this.²³

Relationship Between OVOLs Expression and the Clinical Stage of BRCA

As shown in [Figure 2](#), the OVOL1 expression in BRCA tissues was significantly higher than that in normal BRCA tissues (3.167 ± 0.030 vs 2.114 ± 0.103 , $P<0.001$), the OVOL1 expression in BRCA tissues was significantly higher than that in matched normal breast tissues (3.271 ± 0.075 vs 2.111 ± 0.104 , $P<0.001$); the OVOL2 expression in BRCA tissues was significantly higher than that in breast tissues (4.416 ± 0.023 vs 3.271 ± 0.139 , $P<0.001$), the OVOL1 expression in BRCA tissues was significantly higher than that in matched normal breast tissues (4.253 ± 0.060 vs 3.259 ± 0.140 , $P<0.001$); there was no significant difference in OVOL3 between BRCA tissues and normal breast tissues (0.438 ± 0.013 vs 0.0354 ± 0.028 , $P=0.163$); there was no significant difference in OVOL3 between BRCA tissues and matched normal breast tissues (0.363 ± 0.034 vs 0.0354 ± 0.028 , $P=0.069$). OVOL3 was correlated with the clinical stage of BRCA ([Figure 3](#)). The results suggested OVOL3 may be closely related to the development of BRCA. The area under curve (AUC) of OVOLs was 0.757, 0.754, and 0.537, respectively, suggesting that OVOL1/2 could be served as ideal biomarkers to distinguish BRCA from nontumor tissue ([Figure 4](#)).

Relationship Between OVOLs Expression and Survival in BRCA Patients

As shown in [Figure 5](#), the OS of the OVOL1 mRNA high expression group was shorter than that of the OVOL1 low expression group at all time points (HR:

1.48; 95% CI: 1.07–2.04; $P=0.018$). It suggested that OVOL1 was a risk factor of BRCA. The mRNA of OVOL1 can be used as an indicator for predicting OS progression in BRCA.

Variation and Correlation of OVOLs in BRCA Tissues

As shown in [Figure 6A](#), nearly 5% had mutations in OVOLs, of which less than 1% had missense mutations, less than 1% had structural variants, less than 1% had profound deletions, and about 4% had amplifications, based on the 996 breast malignancy samples. More than 4.8% of patients had increased expression of OVOLs ([Figure 6B](#)). Some proteins were closely associated with OVOLs ([Table S1](#)). The results suggested that changes in the expression profile of OVOLs contributed to the development of BRCA.

GO and KEGG Analyses of OVOLs

The results of analysis showed 25 enriched biological processes, mainly including negative regulation of transcription from RNA polymerase II promoter, positive regulation of transcription from RNA polymerase II promoter, intracellular estrogen receptor signaling pathway, transcription, DNA-templated, chromatin remodeling, transcription from RNA polymerase II promoter, beta-catenin-TCF complex assembly, neural tube closure, covalent chromatin modification, ATP-dependent chromatin remodeling and so on ([Table S2](#) and [Figure 7](#)). The 9 enriched molecular functions included RNA polymerase II transcription factor binding, chromatin binding, RNA polymerase II transcription factor activity, sequence-specific DNA binding, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding, Rab GTPase binding, sequence-specific DNA binding, RNA polymerase II core promoter proximal region sequence-specific DNA binding, NAD-dependent histone deacetylase activity (H3-K14 specific), RNA polymerase II regulatory region sequence-specific DNA binding and so on ([Table S2](#) and [Figure 7](#)). The results of analysis showed 7 cellular components, which were mainly related to nucleus, nuclear chromatin, transcription factor complex, nucleoplasm, intracellular transport particle A, azurophil granule lumen, and Iml1 complex ([Table S2](#) and [Figure 7](#)). The analysis of these functions provides further insight into the cellular localization, geometric distribution, and functional classes of the

Analysis Type by Cancer	OVOL1		OVOL2		OVOL3	
	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal
Bladder Cancer	1		2			
Brain and CNS Cancer						
Breast Cancer	2		8			
Cervical Cancer		1				
Colorectal Cancer						
Esophageal Cancer		2				
Gastric Cancer	1					
Head and Neck Cancer		2	1			
Kidney Cancer		2		1		
Leukemia		1		1		
Liver Cancer						
Lung Cancer	2					
Lymphoma		1				
Melanoma		1		1		
Myeloma				1		
Other Cancer	1			2		
Ovarian Cancer			1			
Pancreatic Cancer						
Prostate Cancer						
Sarcoma				2		
Significant Unique Analyses	7	10	12	7		
Total Unique Analyses	387		398		0	



Figure 1 The mRNA expression of OVOLs between cancer and normal tissues. Cell color is determined by the best gene rank percentile for the analyses within the cell. Red indicates an increase in expression, blue indicates a decrease in expression.

Table 1 OVOLs Expression Differences Between BRCA and Breast Tissues in ONCOMINE Database

OVOLs	Types of Ovarian Cancer vs Ovarian	P value	t-test	Fold Change
OVOL1	Invasive Ductal and Lobular Carcinoma vs Normal	4.12 E-5	5.695	3.447
OVOL1	Mixed Lobular and Ductal Breast Carcinoma vs Normal	8.81 E-5	4.433	2.8535
OVOL2	Invasive Ductal and Lobular Carcinoma vs Normal	2.05 E-8	6.262	3.279
OVOL2	Intraductal Cribriform Breast Adenocarcinoma vs Normal	4.34 E-7	5.543	3.638
OVOL2	Mixed Lobular and Ductal Breast Carcinoma vs Normal	1.75 E-5	4.843	3.091
OVOL2	Tubular Breast Carcinoma vs Normal	2.22 E-25	11.879	2.459
OVOL2	Invasive Ductal and Invasive Lobular Breast Carcinoma vs Normal	2.01 E-28	12.674	2.702
OVOL2	Mucinous Breast Carcinoma vs Normal	3.33 E-14	8.523	2.369
OVOL2	Invasive Lobular Breast Carcinoma vs Normal	8.23 E-28	12.444	2.578
OVOL2	Invasive Breast Carcinoma vs Normal	9.67 E-5	4.320	2.168
OVOL3	NA	NA	NA	NA

OVOLs. KEGG analysis showed that 3 pathways including axon guidance, thyroid hormone signaling pathway, and ubiquinone and other terpenoid-quinone biosynthesis in BRCA were associated with OVOLs (Table S2 and Figure 8). These results helped to understand the molecular mechanisms of OVOLs in the development of BRCA

and provide some theoretical basis for clinical targeted therapy.

Discussion

This study used bioinformatics to investigate the relationship between OVOLs and the development and

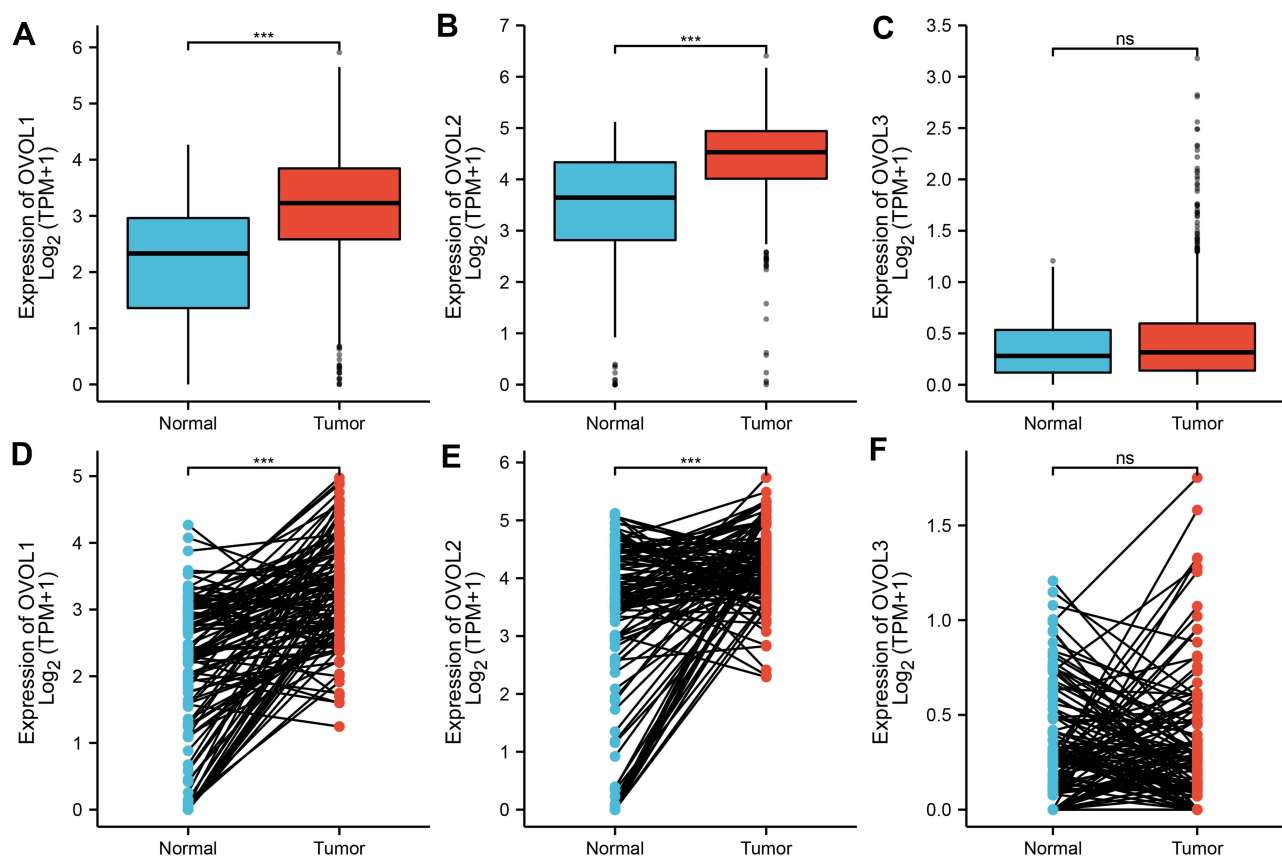


Figure 2 The expression of OVOLs in normal breast tissue and that of BRCA tissues. The difference expression of (A) OVOL1, (B) OVOL2, and (C) OVOL3 in BRCA tissues and normal breast tissues. The difference expression of (D) OVOL1, (E) OVOL2, and (F) OVOL3 in BRCA tissues and paired normal breast tissues. Significance markers: ns, $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

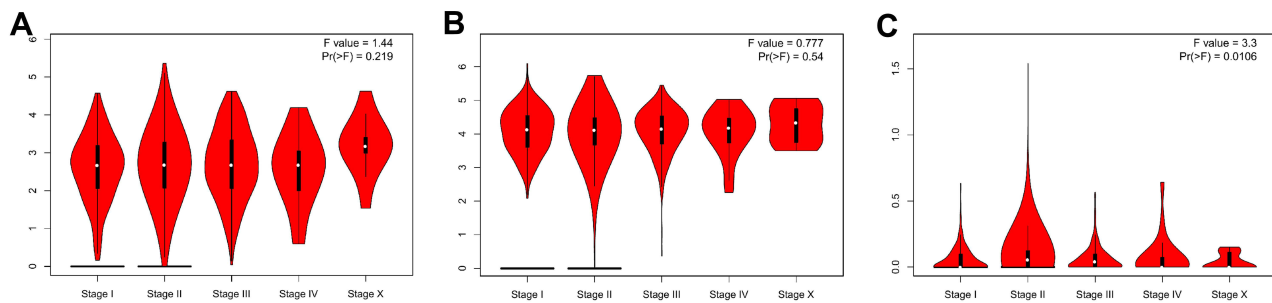


Figure 3 GEPIA of OVOLs in BRCA patients at different tumor stages. (A) OVOL1, (B) OVOL2, and (C) OVOL3.

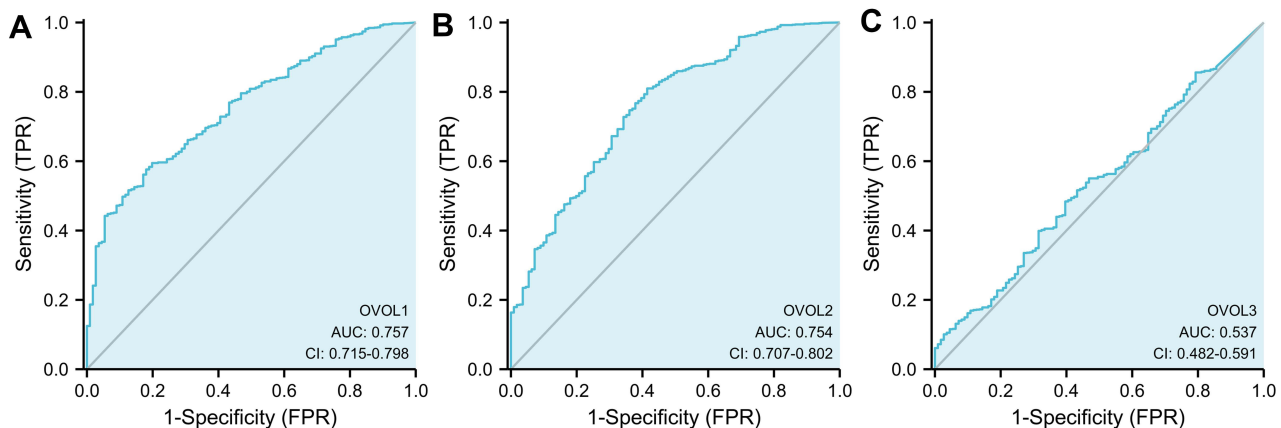


Figure 4 ROC curve showed the efficiency of OVOLs expression level in distinguishing BRCA tissue from nontumor tissues. (A) OVOL1, (B) OVOL2, and (C) OVOL3 were analyzed in this study.

prognosis of BRCA. The results suggest that members of OVOLs could be used as new therapeutic targets and predictive markers for BRCA. OVOLs dysregulation has been reported in many cancers.

OVOL1 was a prognostic predictive marker for oral squamous cell carcinoma (OSCC).¹⁶ OVOL1 was significantly downregulated in cutaneous squamous cell carcinoma.²⁴ OVOL1 expression was evident in eccrine poroma and hidradenoma.²⁵ Low levels of OVOL2 were associated with low overall survival in patients with nasopharyngeal carcinoma (NPC), and could be used as a prognostic indicator for NPC patients.¹⁷ OVOL2 protein overexpression was associated with clinical grade ($P=0.02$) and the recurrence and metastasis ($P=0.02$) of osteosarcoma (OS).¹² OVOL2 expression correlated with clinical staging of lung adenocarcinoma (LUAD).¹³ Overall survival was

significantly lower in patients with hepatocellular carcinoma (HCC) with low OVOL2 expression.²⁶ However, the relationship between OVOL3 and cancer is unclear. In this study, the mRNA expression levels of OVOL1/2 in BRCA tissues were significantly higher than those in normal breast tissues. OVOL3 was correlated with the clinical stage of BRCA. ROC analysis results suggested that OVOL1/2/3 could be served as ideal biomarkers to distinguish BRCA from nontumor tissue. The OVOL1 high expression was associated with OS shortening.

Overexpression of OVOL1 promoted OSCC progression by inhibiting ZEB1.¹⁶ OVOL1 was an upstream inhibitor of c-Myc and OVOL2, and the OVOL1-OVOL2 axis is a regulator of c-Myc that coordinately regulates the aggressiveness of cutaneous squamous cell carcinoma.²⁴ Transcription factors OVOL1 and OVOL2 induce

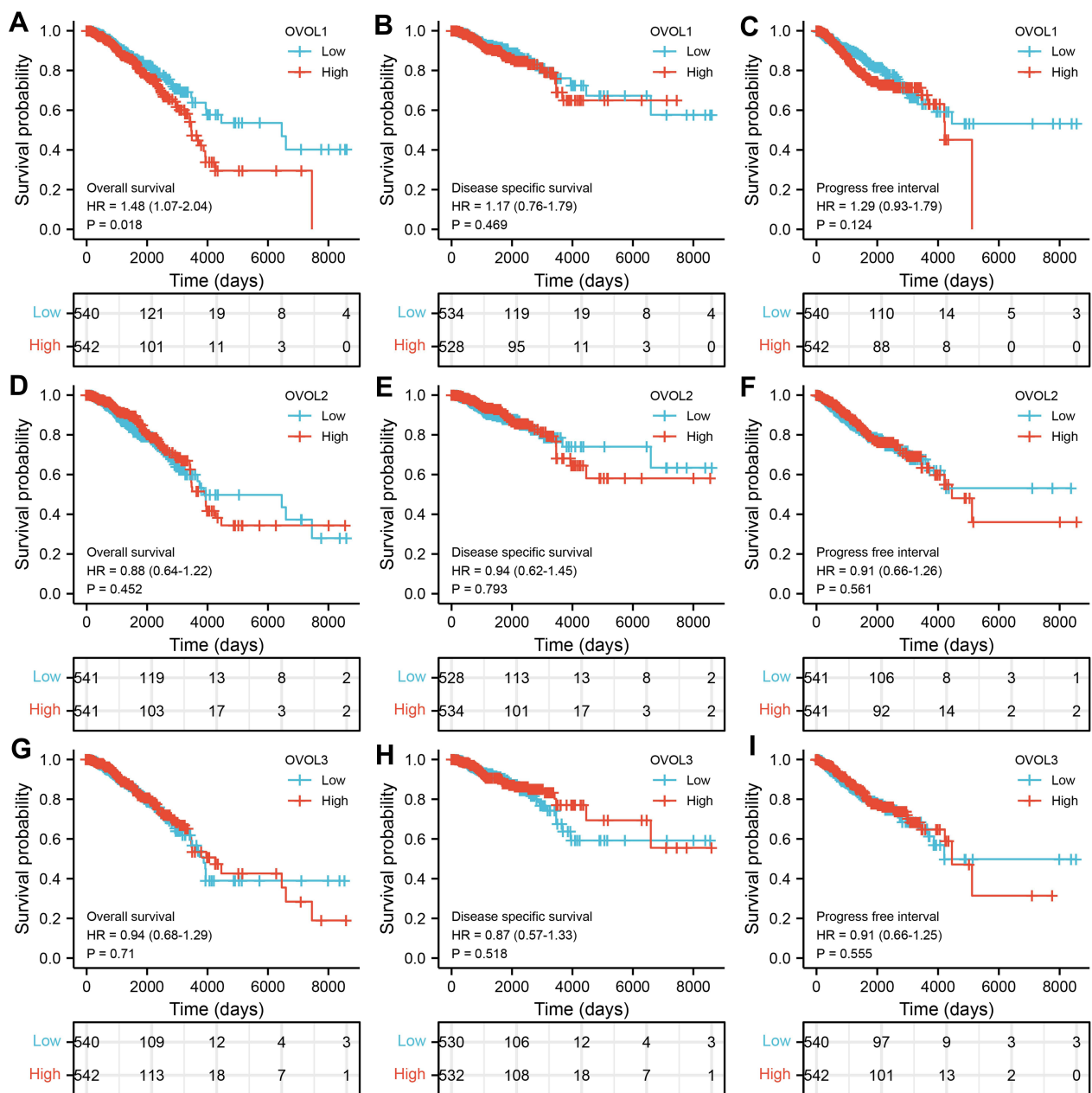


Figure 5 The expression of OVOLs is associated with poor OS in patients with BRCA. Overall survival in BRCA for (A) OVOL1, (D) OVOL2, and (G) OVOL3. Disease-specific survival in BRCA for (B) OVOL1, (E) OVOL2, and (H) OVOL3. Progress free survival in BRCA for (C) OVOL1, (F) OVOL2, and (I) OVOL3.

Epithelial-to-Mesenchymal Transition (EMT) in human cancers.¹¹ PARP1-induced PARYlation was a key event in OVOL2-mediated regulation of chromosome integrity and inhibition of cancer cell growth.²⁷ OVOL2 expression was associated with MET in OS cells and inhibited ZEB1 expression and OS progression.¹² OVOL2 played a key role in inhibiting NPC metastasis and maintaining the epithelial phenotype.¹⁴ OVOL2

inhibited the migratory and invasive capacity of A549 cells and directly prevents EMT by inhibiting Twist1 transcription.¹³ OVOL2 antagonized TGF- β signaling in the regulation of EMT during BRCA metastasis.²⁸ OVOL2 was a colorectal cancer (CRC) suppressor that blocks WNT signaling by promoting the recruitment of histone deacetylase 1 to the TCF4- β -catenin complex.²⁹ Elevated OVOL2 expression may



Figure 8 KEGG analysis of OVOLs co-expression genes predicted by DAVID.

of BRCA prognosis. Further studies are needed to validate these results.

Data Sharing Statement

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declared that they have no conflicts of interest.

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