INTRODUCTION

Worldwide estimates have indicated that breast cancer was the most commonly diagnosed cancer (11.6% of 18.1 million new cases) and the fifth leading cause of cancer mortality (6.6% of 9.6 million deaths) during 2018. Breast cancer is also the most common cancer and the leading cause of cancer deaths among women worldwide. In North America and the European Union, the mortality rate from breast cancer has decreased during recent years due to improvements in early detection and systemic therapies. However, in less developed countries, breast cancer still has the highest mortality rate among all cancers. Lifestyle changes, lack of early detection, and fewer screening programs are the main reasons for the continuing high incidence and mortality of breast cancer in South America, Africa, and Asia. Because early-stage breast cancer is potentially curable, identification of new biomarkers for diagnosis or to guide therapy may reduce mortality rates.

Most human cancers originate from epithelial cells, and polarity proteins play crucial roles in maintaining epithelial structure and function. Cell adhesion and cell polarity complexes (including the crumbs [CRB] complex, partition defective [PAR] complex, and scribble [SCRIB] complex) play key roles in maintaining epithelial cell...
polarity. In mammals, the CRB complex consists of CRB1-3, a protein associated with Lin seven 1 (PalS1), and PALS1-associated tight junction protein (PATJ). The PAR complex consists of PAR3 and 6, and atypical protein kinase C (aPKC). The SCRIB complex consists of scribble, lethal giant larvae (LGL), and discs large (DLG). Abnormal expression of cell adhesion and cell polarity proteins is associated with tumour progression and invasiveness. Recent research indicated alterations in the expression of many cell polarity proteins in human cancers. For example, there is increased expression of PAR6 and aPKC in breast cancer, non-small cell lung cancer, and ovarian cancer. In contrast, CRB3, PAR3, LGL2, DLG1, and DLG5 function as tumour suppressors in many cancers.

Discs large homolog 3 (DLG3), also known as SAP102 (synapse-associated protein 102), is a mammalian homolog of Drosophila DLG tumour suppressor. DLG3 is also in the membrane-associated guanylate kinase (MAGUK) superfamily, whose members contain several PDZ (PSD-95/DLG/ZO-1) domains, an src homology 3 (SH3) domain, and a region with high similarity to guanylate kinases (GK). MAGUK proteins regulate epithelial cell polarity, synaptic development, and synaptic plasticity. Directed trafficking of DLG3 plays important roles in different polarized cell types and in the establishment and maintenance of apical cell junctions and tight junctions of epithelial cells and neuronal synapses. The level of DLG3 mRNA is greater in cancerous breast, kidney, liver, lung, and ovarian tissues than in normal tissues.

In this study, we first analyzed the association of DLG3 expression with the pathological features and probability of survival from breast cancer in The Cancer Genome Atlas (TCGA) database using the UALCAN web tool (http://ualcan.path.uab.edu/index.html). The results indicate that DLG3 expression was significantly greater in breast cancer tissues ($P = 1.62 \times 10^{-12}$) (Figure 1).

### 2.1 Association of DLG3 expression with breast cancer

We initially used the UALCAN web tool (http://ualcan.path.uab.edu/index.html) to compare DLG3 mRNA expression in adjacent normal tissue and breast cancer tissues ($P = 1.62 \times 10^{-12}$). Box plots were produced by UALCAN (http://ualcan.path.uab.edu/index.html)

![Expression of DLG3 mRNA in adjacent normal tissue and breast cancer tissues](image1.png)

**FIGURE 1** Expression of DLG3 mRNA in adjacent normal tissue and breast cancer tissues ($P = 1.62 \times 10^{-12}$). Box plots were produced by UALCAN (http://ualcan.path.uab.edu/index.html)

### 2.2 Association of DLG3 expression with pathological features of breast cancer

Next, we analyzed the association of DLG3 expression with the pathological features of breast cancer using the LINKEDOMICS web tool (http://www.linkedomics.org/login.php). The results indicate that DLG3 correlated with pathologic stage ($n = 1071, P = 1.19 \times 10^{-2}$), with cancer subtype ($n = 826, P = 1.33 \times 10^{-25}$), and with race/ethnicity ($n = 997, P = 4.88 \times 10^{-2}$; Figure 2). Further analysis of Asian patients indicated DLG3 expression was greater in those with more advanced pathologic stage and with the luminal A/B and Her2+ subtypes.

### 2.3 Association of DLG3 expression with survival

Our analysis of the TCGA database using the UALCAN web tool indicated there was a significant association of DLG3 expression with the overall mortality of breast cancer patients (Figure 3A, $P = 3.40 \times 10^{-5}$). In addition, separate analysis of women with three different breast cancer subtypes (luminal A/B, Her2+, and triple-negative) indicated high DLG3 expression was associated with reduced survival time in all three groups (Figure 3B, $P = 6.70 \times 10^{-5}$).

### 2.4 IHC of DLG3 expression

We next used IHC staining to confirm the association of DLG3 expression with pathological features of breast cancer from the TCGA datasets (Figure 4, Table 1). The results indicated that DLG3 expression was highest in stage 3 cancer tissues, and lowest in stage 1 cancer tissues (Figure 4A,C). Further analysis of breast cancer subtypes indicated DLG3 expression was greater in the luminal A/B and Her2+ subtypes than in the triple-negative subtype (Figure 4B,D). Both in the luminal A/B or Her2+ subtypes and in the triple-negative subtypes indicated DLG3 expression was greater in the luminal A/B and Her2+ subtypes than in the triple-negative subtype (Figure 4B,D).
subtype, there is a positive correlation of DLG3 expression with pathological stage (Figure 4E, F).

2.5 | Association of DLG3 expression with survival

We also examined the association of DLG3 expression with survival rate by extraction of survival data of patients whose tissue microarrays were examined. The results of the univariate analysis, using Kaplan-Meier analysis and the log-rank test, indicated that high DLG3 expression was significantly associated with shorter overall survival (OS; Figure 5A, $P = 1.20 \times 10^{-2}$). Separate analysis of the luminal A/B and Her2+ subtypes also indicated high DLG3 expression was associated with reduced OS (Figure 5B, $P = 1.50 \times 10^{-2}$). There was no such association for the triple-negative subtype ($P = .29$, Figure 5C), although we only analyzed data for 29 triple-negative patients. Taken together, the results from our analysis of the TCGA database and tissue microarrays indicate that the level of DLG3 expression correlates with the presence of breast cancer, with more advanced cancer, and with poor OS from breast cancer.

3 | DISCUSSION

Breast cancer is the most common and most lethal of cancers among women worldwide. New tools for the diagnosis and prediction of prognosis may enable earlier onset of treatment and reduce mortality from breast cancer. Many cell polarity proteins have known roles in breast cancer and several other malignancies. Previous research indicated that the levels of DLG3 (a cell polarity protein also named SAP102) were higher in cancers of the breast, kidney, liver, lung, and ovary. Here, we found higher DLG3 expression in cancerous breast tissues than healthy breast tissues and in luminal A/B and Her2+ subtypes than in the triple-negative subtype by using data on clinicopathological parameters from the TCGA database. We also found that high expression of DLG3 was associated with poor prognosis. There were several

**Figure 2** Association of DLG3 expression with (A) breast cancer pathological stage ($n = 1071$, $P = 1.19 \times 10^{-2}$, Kruskal-Wallis test), (B) cancer subtype ($n = 826$, $P = 1.33 \times 10^{-25}$, Kruskal-Wallis test), and (C) race/ethnicity ($n = 997$, $P = 4.88 \times 10^{-3}$, Kruskal-Wallis test). Box plots were produced using LinkedOmics (http://www.linkedomics.org/login.php).

**Figure 3** Association of DLG3 expression with OS of (A) breast cancer patients ($P = 3.40 \times 10^{-4}$) and (B) with OS of patients with different breast cancer subtypes ($P = 6.70 \times 10^{-5}$). Kaplan-Meier curves were produced using UALCAN (http://ualcan.path.uab.edu/index.html). A, Expression level: High expression ($n = 271$); Low/medium expression $n = 810$; B, Expression level, cancer type: Luminal: High ($n = 146$); Low ($n = 408$); Her2+: High ($n = 11$); Low ($n = 26$); Triple negative: High ($n = 16$); Low ($n = 100$).
limitations of this study. First, because this study had a retrospective design, we can only identify associations, and cannot infer casualties. Second, there was no correction for confounding, so any reported association may be due to an unknown intervening factor(s). Nonetheless, we confirmed an association of DLG3 expression with the pathological features of breast cancer by IHC staining of breast cancer tissue microarrays and according to analysis of the TCGA database.

Many proteins in the MAGUK superfamily function in the maintenance of epithelial polarity and cell junctions, and abnormal expression of these proteins is associated with tumour progression. For example, DLG1 has decreased expression in some cervical and breast cancers, a new isoform of DLG2 has increased expression in renal oncocytoma, and ZO1 has decreased expression in breast cancer. The present study suggests that DLG3 may function as an oncogene in breast cancer, although it also appears to act as a tumour suppressor in papillary thyroid carcinoma and glioblastoma. The mammalian DLG3 protein is structurally similar to the Drosophila protein DLG A, which functions as a tumour suppressor in many types of cancers. DLG3 overexpression induces mitotic cell cycle arrest and apoptosis, and inhibits cell proliferation and migration, but has no effect on cell invasion in glioblastoma. Other studies showed that DLG3 inhibited the growth and adhesion of cells by regulating β-catenin in an APC-independent manner. DLG3 also appears to function in coupling NMDA receptors to the MAPK/ERK pathway. Further research is needed to determine whether DLG3 functions in the progression of human malignant tumours via regulation of the MAPK/ERK pathway. DLG3 and several other polarity proteins are overexpressed in different cancers. For example, PAR6 is overexpressed in ER-positive breast cancer and non-small-cell lung cancer; αPKC and PKCζ are overexpressed in hepatocellular carcinoma, bladder tumours, and pancreatic cancer. The results of these studies suggest that overexpression of polarity proteins may be a compensatory mechanism used to establish or maintain proper cell polarity. Thus, the relationship of overexpressed DLG3 in cell polarity maintenance and malignant process of breast cancer needed to be studied in further research.

Previous research predicted that DLG3 is a secreted or plasma membrane protein, and the serum level has potential for use as a diagnostic tool. However, further research is needed to assess the clinical applications of DLG3. Interestingly, up-regulation of miR-1246 reduces DLG3 expression, so microRNA inhibition
seems to be a promising approach to lower the expression of DLG3 in luminal A/B and Her2+ breast cancers.

In conclusion, DLG3 has higher expression in cancerous breast tissues than healthy breast tissues and higher expression in cancers with the luminal A/B and Her2+ subtypes than the triple-negative subtype. DLG3 expression also had a positive correlation with pathologic stage. We suggest that DLG3 might be useful as a diagnostic and prognostic indicator for breast cancer.

4 | MATERIALS AND METHODS

4.1 | Association of DLG3 mRNA with overall survival

ualcan (http://ualcan.path.uab.edu/index.html) is a web tool used to analyze tumour transcriptome data. This web tool provides publicly accessible cancer transcriptome data (TCGA mRNA sequencing), with graphs and plots of gene expression, and information on patient survival. DLG3 mRNA expression in adjacent normal tissue and cancerous breast tissue specimens and Kaplan-Meier survival curves were compared using the ualcan web tool.

| Expression level | N  | High | Low | $\chi^2$ | P    |
|------------------|----|------|-----|--------|------|
| Tumour stage     | 155| 27.09| .0001|
| Stage 1          | 35 | 14   | 21  |        |      |
| Stage 2          | 74 | 19   | 55  |        |      |
| Stage 3          | 46 | 34   | 12  |        |      |
| Breast cancer subtype | 155| 8.44| 3.70 × 10⁻³|
| Luminal A/B & Her2+ | 98 | 51   | 47  |        |      |
| Triple-negative  | 57 | 16   | 41  |        |      |

4.2 | Association of DLG3 expression with pathological features

linkedOmics (http://www.linkedomics.org/login.php) is a web tool used to analyze the multi-omics data from all 32 TCGA cancer types. linkedOmics contains three analytical modules for identification and analysis of data on mRNA or protein expression signatures, biomarkers of clinical attributes, and putative target genes of transcriptional factors, microRNAs, and protein kinases. The association of DLG3 expression with pathological features in breast cancer was analyzed using linkedOmics.31

4.3 | Tissue microarrays, patients, and follow-up

This study was approved by the Ethics Committee on Human Research of the First Affiliated Hospital of Xi’an Jiaotong University (Shaanxi, China). Tissue microarrays of 155 breast cancer tissues were obtained from Shanghai Biochip Co. (Shanghai, China). These samples were from patients with a mean age of 54 years (range: 29–87 years), and 121 of them were followed up for 26–131 months. The last follow-up data were collected from January 2005 to August 2016. The OS time was defined as the time from surgery to death or the last known follow-up. Ninety-five patients were still alive at the last follow-up.

4.4 | Immunohistochemistry (IHC) and scoring

Paraffin-embedded tissue microarray slides were deparaffinized in xylene, rehydrated in ethanol, washed with phosphate-buffered saline (PBS), and a 3% H₂O₂ solution was then used to block endogenous peroxidase activity. After antigen retrieval, goat serum was added to block non-specific binding sites, and the slides were incubated with the anti-DLG3 primary antibody (Proteintech, Wuhan, China) in a moist box at 4°C overnight. The slides were then rinsed, stained using the 3, 3’-diaminobenzidine (DAB) liquid chromogen substrate, counterstained with hematoxylin, and visualized using a Leica Microsystems slide scanner (SCN 400; Leica, Mannheim, Germany). Three randomly selected microscopic fields were

![Figure 5](image-url)
individually examined by blinded investigators in each IHC sample, and the intensity of staining was scored as 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The extent of IHC staining was then assessed according to the percentage of positive cells: 0 (<10%), 1 (10%–40%), 2 (40%–70%) and 3 (>70%). Then the IHC staining score of each field calculated as the product of the intensity and the extent of staining (range, 0–9). Expression was then scored as low (<5) or high (≥5).

4.5 | Statistical analysis

Statistical analyses were performed using GraphPad Prism Version 7.00 (GraphPad, San Diego, CA, USA). The significance of a difference between two groups was determined using the Mann–Whitney test, and the significance of a difference among four groups was determined using the Kruskal–Wallis test. The significance of a difference in the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the log-rank test. For the tissue microarrays, the significance of the difference in the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test. For the tissue microarrays, the significance of the survival rates of two groups was determined using the log-rank test.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (81703002, 81702631 and 81672876).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

ORCID

Peijun Liu https://orcid.org/0000-0003-0529-387X

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7-30.
2. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin. 2017;67:439-448.
3. Harbeck N, Gnant M. Breast cancer. Lancet. 2017;389:1134-1150.
4. Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nat Rev Cancer. 2011;12:23-38.
5. Ellenbroek SL, Iden S, Collard JG. Cell polarity proteins and cancer. Semin Cancer Biol. 2012;22:208-15.
6. Rejon C, Al-Masri M, McCaffrey L. Cell polarity proteins in breast cancer progression. J Cell Biochem. 2016;117:2215-23.
7. Bazzoun D, Lelievre S, Talhouk R. Polarity proteins as regulators of cell junction complexes: implications for breast cancer. Pharmacol Ther. 2013;138:418-27.
8. VanderVorst K, Hatakeyama J, Berg A, Lee H, Carraway KL 3rd. Cellular and molecular mechanisms underlying planar cell polarity pathway contributions to cancer malignancy. Semin Cell Dev Biol. 2018;81:78-87.
9. Li P, Mao X, Ren Y, Liu P. Epithelial cell polarity determinant CRB3 in cancer development. Int J Biol Sci. 2015;11:31-7.
10. Spaderna S, Schmalhofer O, Wahlbuhl M, et al. The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. Cancer Res. 2008;68:537-44.
11. Sandoval GJ, Graham DB, Gmyrek GB, et al. Novel mechanism of tumor suppression by polarity gene discs large 1 (DLG1) revealed in a murine model of pediatric B-ALL. Cancer Immunol Res. 2013;1:426-37.
12. Liu J, Li J, Li P, et al. Loss of DLG5 promotes breast cancer malignancy by inhibiting the Hippo signaling pathway. Sci Rep. 2017;7:42125.
13. Xu B, Krishnamurthy K, Alreed DC, Muthuswamy SK. Loss of Par3 promotes breast cancer metastasis by compromising cell-cell cohesion. Nat Cell Biol. 2013;15:189-200.
14. Smith SA, Holik P, Stevens J, et al. Isolation of a gene (DLG3) encoding a second member of the discs-large family on chromosome 17q12-q21. Genomics. 1996;31:145-50.
15. Stathakis DG, Lee D, Bryant PJ. DLG3, the gene encoding human neuroendocrine Dlg (NE-Dlg), is located within the 1.8-Mb dystonia-parkinsonism region at Xq13.1. Genomics. 1998;49:310-3.
16. Thomas U, Phannavong B, Muller B, Garner CC, Gundelfinger ED. Functional expression of rat synapse-associated proteins SAP97 and SAP102 in Drosophila dlg-1 mutants: effects on tumor suppression and synaptic bouton structure. Mech Dev. 1997;62:161-74.
17. Caruana G. Genetic studies define MAGUK proteins as regulators of epithelial cell polarity. Int J Dev Biol. 2002;46:511-8.
18. Zheng CY, Seabold GK, Horak M, Petralia RS. MAGUKs, synaptic development, and synaptic plasticity. Neuroscientist. 2011;17:493-512.
19. Hanada N, Makino K, Koga H, et al. NE-dlg, a mammalian homolog of Drosophila dlg tumor suppressor, induces growth suppression and impairment of cell adhesion: possible involvement of down-regulation of beta-catenin by NE-dlg expression. Int J Cancer. 2000;86:480-8.
20. Kakunaga S, Ikeda W, Itoh S, et al. Nectin-like molecule-1/TSLL1/SynCAM3: a neural tissue-specific immunoglobulin-like cell-cell adhesion molecule localizing at non-junctional contact sites of presynaptic nerve terminals, axons and glia cell processes. J Cell Sci. 2005;118:1267-77.
21. Wu TH, Chu LJ, Wang JC, et al. Meta-analytical biomarker search of EST expression data reveals three differentially expressed candidates. BMC Genom. 2012;13(Suppl 7):S12.
22. Lin HT, Steller MA, Aish L, Hanada T, Chishti AH. Differential expression of human Dlg in cervical intraepithelial neoplasias. Gynecol Oncol. 2004;93:422-8.
23. Fuja TJ, Lin F, Osann KE, Bryant PJ. Somatic mutations and altered expression of the candidate tumor suppressors CSNK1 epsilon, DLG1, and EDD/hHYD in mammary ductal carcinoma. Cancer Res. 2004;64:942-51.
24. Zubakov D, Stupar Z, Kovacs G. Differential expression of a new isoform of DLG2 in renal oncocytoma. BMC Cancer. 2006;6:106.
25. Hoover KB, Liao SY, Bryant PJ. Loss of the tight junction MAGUK ZO-1 in breast cancer: relationship to glandular differentiation and loss of heterozygosity. Am J Pathol. 1998;153:1767-73.
26. Garcia-Mata R, Dubash AD, Sharek L, Carr HS, Frost JA, Burridge K. The nuclear RhoA exchange factor Net1 interacts with proteins of the Dlg family, affects their localization, and influences their tumor suppressor activity. Mol Cell Biol. 2007;27:8683-97.

27. Liu Z, Niu Y, Xie M, Bu Y, Yao Z, Gao C. Gene expression profiling analysis reveals that DLG3 is down-regulated in glioblastoma. J Neurooncol. 2014;116:465-76.

28. Cuthbert PC, Stanford LE, Coba MP, et al. Synapse-associated protein 102/dlg3 couples the NMDA receptor to specific plasticity pathways and learning strategies. J Neurosci. 2007;27:2673-82.

29. Nolan ME, Aranda V, Lee S, et al. The polarity protein Par6 induces cell proliferation and is overexpressed in breast cancer. Cancer Res. 2008;68:8201-9.

30. Xu LJ, Jiang T, Zhao W, et al. Parallel mRNA and microRNA profiling of HEV71-infected human neuroblastoma cells reveal the up-regulation of miR-1246 in association with DLG3 repression. PLoS ONE. 2014;9:e95272.

31. Vasaikar SV, Straub P, Wang J, Zhang B. LINKEDOMICS: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46:D956-D963.

How to cite this article: Liu J, Li P, Wang R, et al. High expression of DLG3 is associated with decreased survival from breast cancer. Clin Exp Pharmacol Physiol. 2019;46:937-943. https://doi.org/10.1111/1440-1681.13132