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Identification of endothelial selectin as a potential prognostic marker in breast cancer

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Abstract. Endothelial selectin (*ELAM1* or *CD62E*) has been previously reported as being associated with the prognosis of multiple types of cancer. However, its prognostic value in breast cancer (BC) remains unclear. The aim of the present study was to investigate the prognostic value of *ELAM1* mRNA expression in BC tissue. The prognostic value of *ELAM1* mRNA was assessed in patients with BC using the Kaplan-Meier plotter (KM-plot) database. The KM-plot generated updated *ELAM1* mRNA expression data and survival analysis from a total of 3,951 patients with BC, gathered from 35 datasets. Low expression of *ELAM1* mRNA was correlated with a poorer overall survival in 1,402 patients with BC followed for 20 years [hazard ratio (HR), 0.71; 95% confidence interval (CI), 0.57-0.88; log-rank P=0.0016]. Low expression of *ELAM1* was also correlated with poorer relapse-free survival (HR, 0.69; 95% CI, 0.62-0.77; log-rank P=2.2e-11) in 3,951 patients and poorer distant metastasis-free survival (HR, 0.79; 95% CI, 0.65-0.96; log-rank P=0.02) in 1,746 patients with BC followed for 20 years. Results from the Metabolic gEne RApid visualizer database indicated that *ELAM1* mRNA expression was elevated in normal tissue. The results of the present study suggest that *ELAM1* mRNA is a potential prognostic and metastatic marker in patients with BC.

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Abbreviations: BC, breast cancer; HR, hazard ratio; CI, confidence interval; KM-plot, Kaplan-Meier plotter; OS, overall survival; RFS, relapse-free survival; DMFS, distant metastasis-free survival; PPS, post-progression survival; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor; TP53, tumor protein p53

Key words: breast cancer, ELAM1, prognosis, Kaplan-Meier plotter, marker

Introduction

Breast cancer (BC) is the most common female cancer worldwide and the second leading cause of cancer-related death. Its etiology involves genetic and environmental factors. Metastasis is the major challenge in BC therapy (1). Gene therapy can treat, cure, or prevent a particular disease as the transfer of foreign genetic materials to a patient. Solid tumor tissues can be significantly enhanced the targeting ability of delivery systems to solid tumors (2). Recent studies have reported that mRNA shows high potential in gene therapy (2,3). However, the gene therapy of endothelial selectin (also known as *ELAM1 or CD62E*) mRNA remains unclear in BC.

Surgical resection is potentially curative, but its prognosis is often unpredictable. The evaluation of prognosis in patients with BC who have undergone surgical resection is essential for chemotherapy planning. A major obstacle is the lack of predictive tools capable of estimating post-treatment prognosis (4,5). The results of recent studies have shown that no single method can fully predict prognosis in patients with BC; scholars have made sustained efforts to identify the most useful approaches (6-9), including examination of the predictive value of numerous genes (10,11).

Intercellular adhesion molecules play important roles in tumor progression and metastasis, which have traditionally been regarded as important indicators of tumor prognosis (12-17). These complex processes involve several mechanisms, such as uncontrolled cell growth, intercellular interactions, leukocyte changes, adhesion of vascular endothelium, and the induction of neoangiogenesis (18-20). In BC, serum levels of adhesion molecules have been correlated with tumor progression and metastasis (12-17,21-23).

ELAM1 is a member of the selectin adhesion molecule family with a molecular weight of 97-115 kDa, which is expressed on endothelial cells activated by cytokines (4,6,24,25). It mediates the rolling of neutrophils and leukocytes on the surfaces of endothelial cells. In previous studies, ELAM1 levels were elevated in patients with hepatocellular, prostate (26), renal (27), colon (28), gastrointestinal, and ovarian cancers (29) and BC (19,25,30,31). Most research

to date, however, has focused on soluble ELAM1. The prognostic implications of *ELAM1* mRNA in BC remain unclear.

An online survival analysis tool that can be available to evaluate the prognostic implications of single genes in BC (11,32,33). We used an integrative data analysis tool (http://kmplot.com/) to confirm the predictive values of proliferation-related *ELAM1* genes. Data entered into the Kaplan-Meier plotter (KM-plot) were extracted from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database. At present, the KM-plot database can be used to evaluate the prognostic values of 54,675 genes using 10,461 cancer samples from patients (5,143 breast, 2,437 lung, 1,816 ovarian, 1,065 gastric cancer; mean follow-up periods of 69, 49, 40, and 33 months, respectively). Survival analyses conducted with data on individual genes can be validated by KM-plot, and KM-plot have been utilized by lots of genes in ovarian cancer (34,35), BC (11,36-41), gastric cancer (42), and non-small cell lung cancer (43,44).

In this study, the prognostic value of individual *ELAM1* was assessed in patients with BC using KM-plot.

Materials and methods

Kaplan-Meier survival analysis. An online KM-plot database can be utilized to identify the relevance of individual *ELAM1* mRNA expression in survival analyses, including the examination of overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS), and post-progression survival (PPS). The KM-plot database is handled by a MySQL server, which synchronously integrates gene expression and clinical data. Survival curves are calculated using the 'survival' package, and the number-at-risk is displayed below the main plot. Hazard ratios (HRs), 95% confidence intervals (CIs), number of risk, and log-rank P-values are also indicated on the webpage (11). Number of risk can be interpreted as the number of surviving patients. HR is the ratio of the hazard rates corresponding to the conditions described by two levels of an explanatory variable.

Construction of BC microarray database. The database for this study was constructed using gene expression data and survival information from 3,951 patients with BC followed for 20 years. These data were downloaded from GEO, the Cancer Genome Atlas (https://cancergenome.nih.gov/), the European Genome-Phenome Archive (https://ega.crg.eu/), and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). Briefly, *ELAM1* was entered into Affymetrix ID choosing the probe set 206211_at (*ELAM1*) in the KM-plot database (http://kmplot.com/analysis/index.php?p=service&cancer=breast) to obtain KM-plots. The analysis determines whether high (above the median) and low (below the median) ELAM1 mRNA expression are associated with significantly different prognoses in patients with BC. We then conducted stratified analyses to evaluate correlations with estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor 2 (HER2) status, lymph node status, pathological grade, tumor protein p53 (TP53) status, intrinsic subtype, and Pietenpol subtype in patients with BC.

Cancer and normal tissue analysis. The Metabolic gEne RApid Visualizer (MERAV) website (http://merav.wi.mit.edu/SearchByGenes.html) was developed to provide additional advanced web-based tools for the analysis of gene expression in tumor and normal tissues (45). MERAV is linked to two other databases, the National Center for Biotechnology Information's Entrez Gene database (http://www.ncbi.nlm.nih.gov) and the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) (46), which allows the user to acquire more comprehensive information for each gene selected. We further identified *ELAM1* mRNA expression in BC and normal tissues using the MERAV database. We conducted a search for the *ELAM1* gene in MERAV, which automatically generates boxplots of the data.

Statistical analysis. Univariate survival analyses were conducted using Kaplan-Meier survival curves. HRs with 95% CIs were calculated using a Cox proportional-hazards regression model to evaluate survival ratios. Stratified analyses were conducted to further confirm the correlations of individual *ELAM1* with other clinicopathological features. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Data sources. We identified all together 3,951 patients in the GEO, TCGA, EGA, and PubMed. There were no samples repeatedly published (11). The validation was performed on microarrays which were previously published in following datasets: E-MTAB-365, E-TABM-43, GSE11121, GSE12093, GSE12276, GSE1456, GSE16391, GSE16446, GSE16716, GSE17705, GSE17907, GSE18728, GSE19615, GSE20194, GSE20271, GSE2034, GSE20685, GSE20711, GSE21653, GSE2603, GSE26971, GSE2990, GSE31448, GSE31519, GSE32646, GSE3494, GSE37946, GSE41998, GSE42568, GSE45255, GSE4611, GSE5327, GSE6532, GSE7390 and GSE9195.

Survival analysis. The KM-plot curves showed that low expression of *ELAM1* mRNA was correlated with worse OS in 1,402 patients with BC followed for 20 years (HR=0.71; 95% CI, 0.57-0.88; log-rank P=0.0016; Fig. 1A). Low expression of *ELAM1* mRNA was correlated strongly with worse RFS (HR=0.69; 95% CI, 0.62-0.77; log-rank P=2.2e-11) in 3,951 patients with BC and worse DMFS (HR=0.79; 95% CI, 0.65-0.96; log-rank P=0.02) in 1,746 patients with BC followed for 20 years (Fig. 1B and C).

PPS showed no significant difference in the survival analysis or stratified analysis of 414 patients with BC (Fig. 1D and Table I). Stratified analyses further confirmed the correlations of individual *ELAM1* with other clinicopathological features. *ELAM1* mRNA expression was elevated in normal tissues (Fig. 2).

In the analysis stratified by OS, individual *ELAM1* mRNA expression was associated with pathological grade 2 in 387 patients (HR=0.63; 95% CI, 0.41-0.98; log-rank P=0.038; Table II). No other stratum of OS showed a significant association.

In the analysis stratified by RFS, high expression of *ELAM1* significantly decreased the risk of metastasis among patients with ER positivity (HR=0.65, log-rank

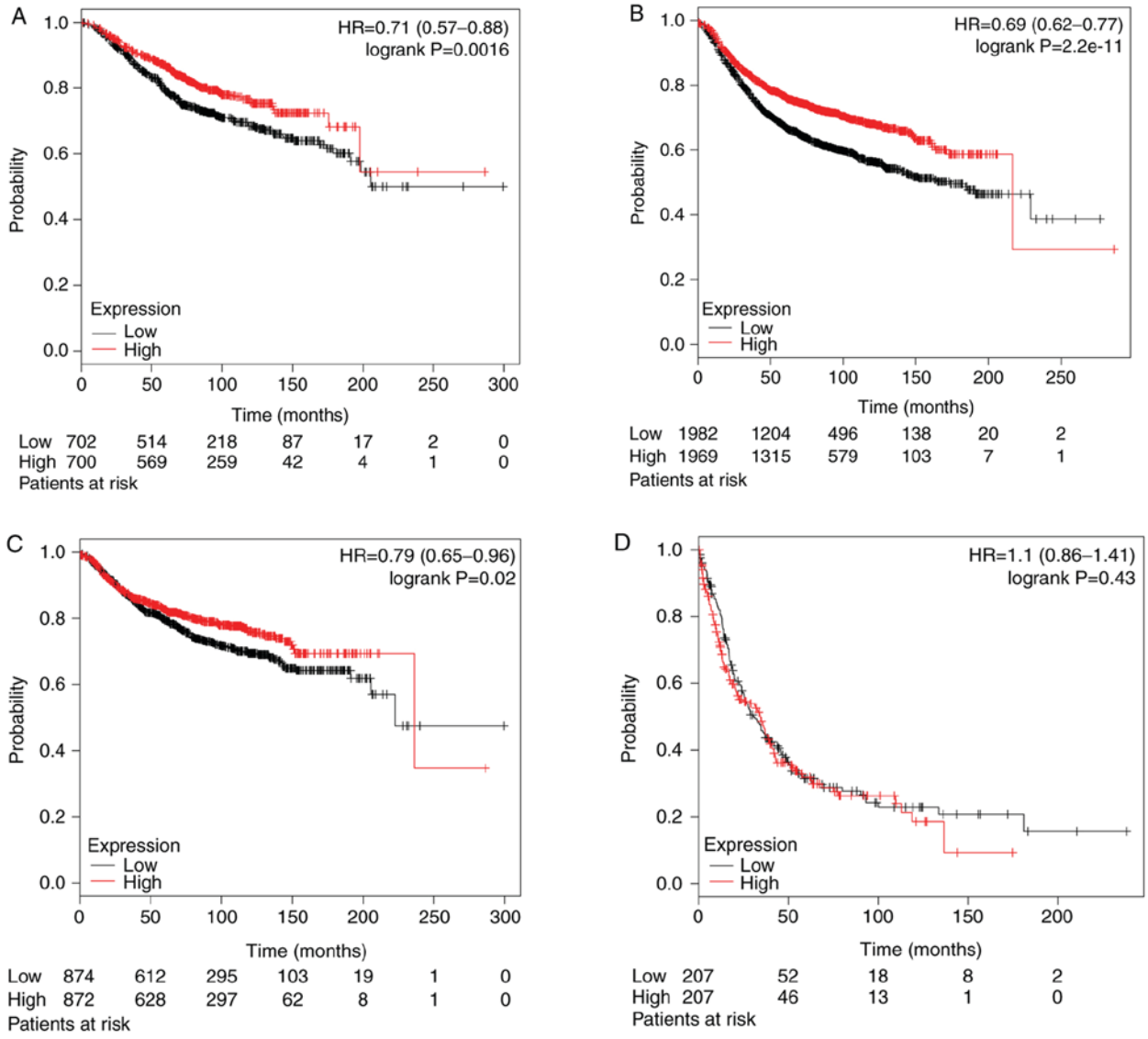

Figure 1. The prognostic value of ELAM1 expression. Curves for (A) overall survival (n=1,402), (B) relapse-free survival (n=3,951), (C) distant metastasis-free survival (n=1,746) and (D) post-progression survival (n=414). The small vertical bars cross the curve indicate censoring. The desired Affymetrix ID is available: 206211_at (ELAM1, also known as CD26E). Data were analyzed using the Kaplan Meier Plotter (http://kmplot.com/). HR, hazard ratio.

P=5.2e-07), ER negativity (HR=0.79, log-rank P=0.041), PR positivity (HR=0.58, log-rank P=0.0029), HER2 negativity (HR=0.53, log-rank P=3.5e-06), lymph node positivity (HR=0.82, log-rank P=0.046), lymph node negativity (HR=0.73, log-rank P=3e-04), pathological grades 2 (HR=0.77, log-rank P=0.034) and 3 (HR=0.76, log-rank P=0.013), the basal subtype (HR=0.67, log-rank P=0.0019), the luminal A subtype (HR=0.64, log-rank P=3.5e-07), the luminal B subtype (HR=0.69, log-rank P=0.0001), the TP53 wild type (HR=0.56, log-rank P=0.0073), and the immunomodulatory subtype (HR=0.56, log-rank P=0.0073; Table III).

Comparison of cancer and normal tissue. The analysis stratified by DMFS demonstrated that low expression of individual *ELAM1* mRNA significantly increased the risk of metastasis in patients with ER positivity (HR=0.67, log-rank P=0.02) and HER2 negativity (HR=0.22, log-rank P=0.0027; Table IV). No other DMFS stratum showed a significant association.

Discussion

Multiple studies have indicated that high expression of ELAM1 is associated with significantly worse OS and an increased risk of metastasis in patients with hepatocellular carcinoma (47), prostate cancer (26), colorectal cancer (28,48,49), and BC (29,50). Research conducted by Zhang and Adachi (51) suggested that soluble ELAM1 expression is a prognostic factor for advanced tumors. However, previous studies examined soluble ELAM1 and/or had *in vitro* designs. *In vivo*, the plasma and serum levels of ELAM1 are influenced by conditions such as diabetes, arthritis, and inflammation (52,53). Multiple studies (19,22,54) have shown that soluble ELAM1 is not a significant prognostic factor for BC metastasis. Muraki *et al* (55) demonstrated that high levels of soluble ELAM1 had an anti-tumoral effect in renal cell carcinoma (RCC) and significantly decreased the risk of RCC metastasis.

In the present study, we assessed the predictive significance of *ELAM1* mRNA in 3,951 patients with BC. Their tumor

Table I. Correlation of endothelial selectin expression with post-progression survival in patients with breast cancer.

Variable	Group	Cases	HR	95% CI	Log-rank P-value
ER status	Positive	173	0.95	0.64-1.42	0.81
	Negative	100	0.74	0.44-1.25	0.26
PR status	Positive	13	N/A	N/A	N/A
	Negative	17	N/A	N/A	N/A
HER2 status	Positive	33	0.85	0.32-2.24	0.74
	Negative	39	1.16	0.43-3.13	0.76
Lymph node status	Positive	128	1.1	0.70-1.72	0.68
	Negative	165	0.97	0.63-1.49	0.89
Grade	1	34	0.81	0.30-2.22	0.69
	2	128	1.18	0.73-1.92	0.49
	3	165	1.01	0.69-1.48	0.96
Intrinsic subtype	Basal	64	0.93	0.52-1.66	0.79
	Luminal A	179	0.99	0.68-1.47	0.98
	Luminal B	134	1.10	0.71-1.69	0.67
	HER2+	37	1.31	0.62-2.76	0.48
TP53 status	Mutated	34	0.68	0.28-1.65	0.39
	Wild type	62	1.31	0.66-2.61	0.43
Pietenpol subtype	Basal-like 1	171	0.90	0.56-1.45	0.66
	Basal-like 2	76	1.09	0.54-2.21	0.81
	Immunomodulatory	17	N/A	N/A	N/A
	Mesenchymal	24	0.95	0.39-2.33	0.91
	Mesenchymal stem-like	4	N/A	N/A	N/A
	Luminal androgen receptor	32	0.46	0.20-1.07	0.06

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TP53, tumor protein p53; N/A, not available.

specimens were analyzed using the probe set 206211_at. We found that high expression of *ELAM1* mRNA was associated significantly with increased OS, RFS, and DMFS in patients with BC, contrary to previous research results for soluble ELAM1 (12-17,19,21,22,25,47,51). The stratified analysis showed that high expression of *ELAM1* mRNA was associated with better OS in patients with grade 2 BC. Low *ELAM1* mRNA expression was correlated with metastasis in ER-positive and HER2-negative patients. Furthermore, the results from the MERAV and KM-plot databases were consistent. The results of previous studies have rarely been reported. Our results show that *ELAM1* mRNA is an anti-oncogene that plays an important role in the evaluation of BC prognosis. Thus, the prognostic values of *ELAM1* mRNA in different tumors differ, which should be kept in mind.

The difference in findings on the association of *ELAM1* mRNA expression and ELAM1 plasma concentration with BC prognosis (21,22,25,29,50,51) is likely attributable to the following factors. Adhesion molecules of activated endothelial cells have dual roles in tumor growth and metastasis (53). They are part of a host immune response, which explains the sustained elevation of serum ELAM1 levels in patients with cancer. Shedding of adhesion

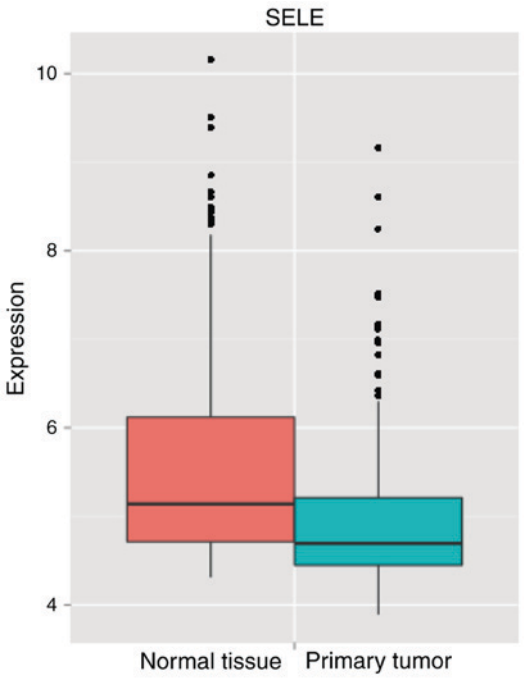

Figure 2. Expression profiles of endothelial selectin in breast cancer and normal tissues, determined using the Metabolic gEne RApid Visualizer.

Table II. Correlation of endothelial selectin expression with overall survival in patients with breast cancer.

Variable	Group	Cases	HR	95% CI	Log-rank P-value
ER status	Positive	548	0.72	0.50-1.03	0.069
	Negative	251	0.72	0.45-1.14	0.160
PR status	Positive	83	0.30	0.06-1.47	0.120
	Negative	89	1.36	0.54-3.44	0.520
HER2 status	Positive	252	0.81	0.52-1.25	0.340
	Negative	130	0.57	0.23-1.42	0.220
Lymph node status	Positive	313	0.76	0.51-1.12	0.160
	Negative	594	0.82	0.57-1.20	0.310
Grade	1	161	0.52	0.21-1.33	0.170
	2	387	0.63	0.41-0.98	0.038
	3	503	0.86	0.62-1.19	0.350
Intrinsic subtype	Basal	241	0.69	0.42-1.14	0.140
	Luminal A	611	0.70	0.49-1.01	0.056
	Luminal B	433	0.73	0.50-1.06	0.095
	HER2+	117	0.77	0.40-1.48	0.440
TP53 status	Mutated	111	0.63	0.29-1.36	0.230
	Wild type	187	0.94	0.49-1.78	0.840
Pietenpol subtype	Basal-like 1	58	0.83	0.28-2.48	0.740
	Basal-like 2	38	2.80	0.72-10.85	0.120
	Immunomodulatory	100	0.78	0.31-1.97	0.590
	Mesenchymal	73	0.64	0.29-1.41	0.270
	Mesenchymal stem-like	19	N/A	N/A	N/A
	Luminal androgen receptor	83	1.01	0.52-1.99	0.970

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TP53, tumor protein p53; N/A, not available.

molecules by activated endothelial cells may possibly serve to 'block' counter ligands, for example on tumor cells, and subsequently prevent their adhesion to endothelial cells at metastatic sites (53). The significance of adhesion molecule shedding is not clear. They can also help adhesion molecules escape from the host defense mechanisms, thereby promoting dissemination and metastasis. Invasive BC cells resist host defense mechanisms only if they are able to survive. Madhavan *et al* (4) suggested that soluble ELAM1 could serve as an endothelial damage marker after activation by cytokines and the prompting of enhanced host defense mechanisms against the tumor. They pointed out that soluble ELAM1 was not a significant risk factor in patients with BC and nodal positivity. They suggested that the prognostic implications of soluble ELAM1 could be identified by survival analysis of long-term follow-up data from patients with BC. Thus, our findings do not conflict with data from previous studies (12,13,17,19,21,22,25,29, 50,51). The mechanism of serum ELAM1 release remains ambiguous, and the prognostic value of this marker is controversial (4,19,22,25,27,28,30,31,51,54,55). An online analysis of tumor-dependent gene expression is essential to clarify the mechanisms involved. Our results confirmed that high *ELAM1* mRNA expression in tumor specimens was a favorable factor for the prognosis of patients with BC.

Treatment has an important effect on the plasma level of ELAM1. Chemotherapeutic agents used for the treatment of BC, including gemcitabine, anthracyclines, and vinca alkaloids (21,56), may have endothelial toxicity and can cause endothelial cell apoptosis or necrosis *in vitro* (57). The plasma ELAM1 concentration is derived from endothelial damage following activation by cytokines. It may increase the serum ELAM1 concentration and interfere with the determination of the correlation between this concentration and BC prognosis. Moreover, intact endothelium is a prerequisite for normal functioning of the host defense system, and endothelial damage results in endothelial dysfunction. These factors may lead to the elevation of serum ELAM1 levels in patients with BC, worsening outcomes. The effects of soluble ELAM1 on host defense mechanisms and the promotion of tumor progression and metastasis are very complex.

ELAM1 is expressed in many cells other than endothelial cells, including lymphocytes, fibroblasts, and hematopoietic cells (22,58,59). The concentration of soluble ELAM1 can be affected by conditions such as diabetes (52), arthritis (53),

Table III. Correlation of endothelial selectin expression with relapse-free survival in patients with breast cancer.

Variables	Group	Cases	HR	95% CI	Log-rank P-value
ER status	Positive	2,061	0.65	0.55-0.77	<0.01
	Negative	801	0.79	0.63-0.99	0.04
PR status	Positive	589	0.58	0.40-0.83	<0.01
	Negative	549	0.79	0.59-1.06	0.12
HER2 status	Positive	252	0.81	0.52-1.25	0.34
	Negative	800	0.53	0.40-0.70	<0.01
Lymph node status	Positive	1,133	0.82	0.67-1.00	<0.05
	Negative	2,020	0.73	0.62-0.87	<0.01
Grade	1	345	0.59	0.34-1.01	0.05
	2	901	0.77	0.61-0.98	0.03
	3	903	0.76	0.61-0.94	0.01
Intrinsic subtype	Basal	618	0.67	0.52-0.86	<0.01
	Luminal A	1,933	0.64	0.54-0.76	<0.01
	Luminal B	1,149	0.69	0.57-0.83	<0.01
	HER2+	251	0.9	0.61-1.32	0.58
TP53 status	Mutated	188	0.77	0.48-1.24	0.28
	Wild type	273	0.56	0.36-0.86	<0.01
Pietenpol subtype	Basal-like 1	171	0.9	0.56-1.45	0.66
	Basal-like 2	76	1.09	0.54-2.21	0.81
	Immunomodulatory	203	0.53	0.29-0.98	0.04
	Mesenchymal	177	0.75	0.49-1.14	0.18
	Mesenchymal stem-like	63	1	0.46-2.20	0.99
	Luminal androgen receptor	203	0.96	0.64-1.44	0.84

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TP53, tumor protein p53.

cigarette smoking (60), chronic inflammatory syndromes (61), systemic infections (62), cardiovascular disease (63), and chronic renal failure (64). Chronic inflammation, an important factor in the development of BC, has been shown to increase endothelial cell proliferation (65). For several reasons, obtaining reproducible correlations of serum ELAM1 levels with BC prognosis has been shown to be difficult (4,19,21,22,25,27,28,30,31,51,54,55). The search for BC gene expression will be an accurate direction for prognosis estimation in the future.

Several limitations of this study must be recognized. Our data from the web-based tool were used only to perform univariate analysis; multivariate survival analysis using a Cox proportional-hazards regression model was not performed because of the incompletes of clinical KM-plot data. However, the prognostic evaluation of individual genes was based on data from 3,951 patients with BC, and the results were consistent with those from the MERAV database. Our results provide insight into the association between *ELAM1* and BC prognosis.

In conclusion the use and development of *ELAM1* mRNA as a predictive factor for BC will definitely benefit clinicians. Further investigation with well-designed studies and large samples is essential to validate our results.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DS designed the research; WZ wrote the manuscript; WZ, ZZ and XH collected the data; JL and GJ analyzed the data, and

Table IV. Correlation of endothelial selectin expression with distant metastasis-free survival in patients with breast cancer.

Variables	Group	Cases	HR	95% CI	Log-rank P-value
ER status	Positive	664	0.67	0.47-0.94	0.02
	Negative	218	0.82	0.52-1.30	0.40
PR status	Positive	192	0.49	0.20-1.19	0.11
	Negative	154	0.73	0.41-1.32	0.30
HER2 status	Positive	126	0.53	0.27-1.03	0.06
	Negative	150	0.22	0.07-0.65	<0.01
Lymph node status	Positive	382	0.84	0.57-1.24	0.38
	Negative	988	0.84	0.64-1.10	0.21
Grade	1	188	0.88	0.38-2.07	0.77
	2	546	0.78	0.55-1.11	0.16
	3	458	1.02	0.72-1.44	0.91
Intrinsic subtype	Basal	232	0.69	0.41-1.15	0.15
	Luminal A	965	0.86	0.64-1.15	0.30
	Luminal B	430	0.7	0.49-1.01	0.05
	HER2+	119	0.84	0.45-1.56	0.57
TP53 status	Mutated	83	1.08	0.45-2.59	0.87
	Wild type	109	0.72	0.33-1.56	0.40
Pietenpol subtype	Basal-like 1	65	0.88	0.34-2.29	0.80
	Basal-like 2	39	1.09	0.41-2.92	0.87
	Immunomodulatory	96	0.49	0.19-1.25	0.13
	Mesenchymal	65	1.06	0.44-2.55	0.89
	Mesenchymal stem-like	17	N/A	N/A	N/A
	Luminal androgen receptor	82	0.46	0.21-1.02	0.05

HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TP53, tumor protein p53; N/A, not available.

GJ modified the manuscript. All authors gave final approval of this submission.

Ethics approval and consent to participate

The study was reviewed and approved by the Affiliated Tumor Hospital of Guangxi Medical University Institutional Review Board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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