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Original Article

High expression of CSE1L is associated with poor prognosis in breast cancer

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Abstract: Cellular apoptosis susceptibility (chromosome segregation 1-like, CSE1L) is a microtubule-associated protein that is highly expressed in various cancers. In this study, CSE1L expression was evaluated in 81 normal tissues and 370 breast cancer tissues via immunohistochemical staining (IHC). The associations between CSE1L expression and clinicopathological features were evaluated. Additionally, Kaplan-Meier analysis and Cox proportional hazards regression models were utilized to determine the correlation between CSE1L expression and prognosis. Our study shows that the nuclear and cytoplasmic expression levels of CSE1L in breast cancer tissues are significantly higher than those in normal tissues. High nuclear CSE1L expression is positively correlated with tumor size (P = 0.014), lymph node metastasis (P = 0.018), stage (P < 0.001) and poorer overall survival (P = 0.011). Furthermore, multivariate Cox regression analysis shows that high nuclear CSE1L expression is an independent prognostic factor in patients with breast cancer (P = 0.039). These results indicate that high CSE1L expression may be a biomarker for breast cancer prognosis.

Keywords: CSE1L, breast cancer, prognosis

Introduction

Breast cancer is the most common malignancy and the second-leading cause of tumor-related mortality among women worldwide [1]. Although early detection and combination treatments have improve significantly, breast cancer therapy and prevention remain major public health concerns. Therefore, there is an urgent need to identify biomarkers for prognosis and therapy responsiveness.

Cellular apoptosis susceptibility (chromosome segregation 1-like, CSE1L), which is located on chromosome 20q13, is the human homolog of the yeast CSE1 gene. CSE1L was originally identified during a screening for genes that were capable of causing MCF-7 human breast cancer cells to evade apoptosis [2, 3]. CSE1L overexpression has been observed in several types of cancer, including melanoma [4], lymphoid neoplasms [5], breast cancer [6], hepatocellular carcinoma [7, 8], ovarian carcinoma [9], colorectal cancer [10, 11] and endometrial carcinoma [12]. CSE1L is associated with microtubules and the mitotic spindle [13]. Mechanistic studies suggest that it may promotes cancer cell proliferation [4-7]. Furthermore, CSE1L depletion significantly increases the proportions of apoptotic cells [10], and reduces cancer cell migration ability [14]. It has also been shown that high levels of CSE1L expression are correlated with poor overall survival [7, 9, 10]. These findings suggest that CSE1L plays an important role in cancer progression. Hence, we undertook this study to investigate CSE1L expression in breast cancer and to determine the relationship between CSE1L expression and other clinicopathological variables.

Materials and methods

Patients and tissue samples

A total of 370 Chinese breast cancer samples were collected after obtaining informed consent from the Harbin Medical University Cancer Hospital. All the patients underwent surgery in 2006. None of the patients received chemotherapy or radiotherapy before surgery. All tis-

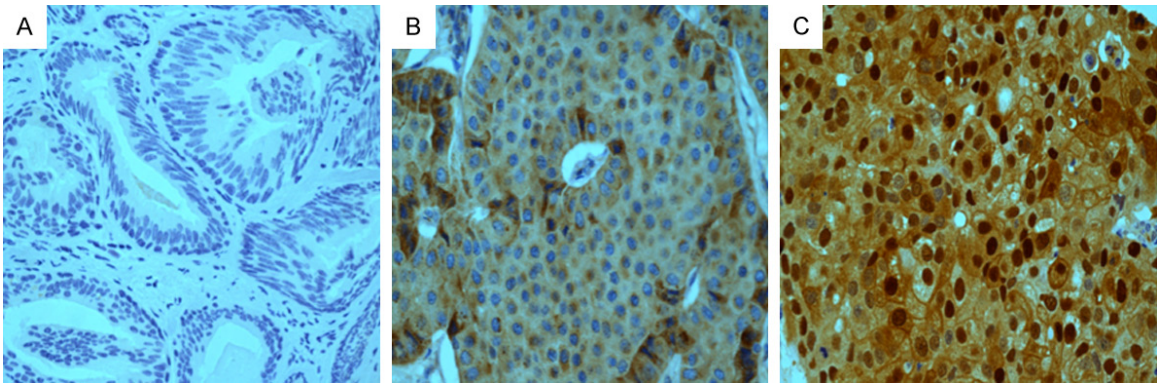

Figure 1. Expression of CSE1L protein in tissue by immunohistochemistry. A. Cytoplasmic and nuclear low expression of CSE1L in benign breast specimen. B. Cytoplasmic high expression of CSE1L in breast cancer specimen. C. Cytoplasmic and nuclear high expression of CSE1L in breast cancer specimen (400 ×).

sues were verified by two pathologists on the basis of pathological testing. Clinicopathological classification and staging were determined according to the American Joint Committee on Cancer (AJCC) criteria. Expression of ER, PR and HER2 was assessed according to the American Society of Clinical Oncology/College of American Pathologists guidelines.

Tissue microarray (TMA)

The breast cancer TMA was processed as follows. Briefly, a tissue array instrument was used to create holes in a receptive paraffin block and to acquire tissue cores from the donor tissue block using a thin-walled needle with an inner diameter of 2 mm that was held in an X-Y precision guide. The core samples were retrieved from the selected region in the donor block and extruded directly into the receptive block at defined array coordinates. A solid steel wire that was closely fitted in the tube was used to transfer the tissue cores into the receptive block. After the construction of the array block, all the tissue blocks were cut with a microtome to 4 μm and affixed to slides. Blocks from 370 patients' surgical breast carcinomas and corresponding normal breast sample were arrayed as triplicate spots of 2 mm in diameter on the slides.

Immunohistochemistry (IHC)

Immunohistochemical staining was carried out following the protocol of our previous study [15]. The rabbit polyclonal antibody against CSE1L (1:100; Abcam) was added to the sections for overnight at 4°C, followed by incubation with rabbit secondary antibody for 30 minutes at room temperature. After washing in PBS, each section was treated with diaminobenzadine at room temperature, and then washed in distilled water.

Immunohistochemistry analysis was performed by two pathologists. The percentage of immunoreactive cells was combined with the estimated staining intensity. The staining intensity score varied from 0 to 3 (0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining). The total possible intensity was calculated using the following formula: (0 × percentage of unstained tumor cells) + (1 × percentage of weakly stained tumor cells) + (2 × percentage of weakly stained tumor cells) + (3 × percentage of strongly stained tumor cells). Finally, the staining results were categorized into low CSE1L expression (CSE1L staining 0 and 1+) and high CSE1L expression (CSE1L staining 2+ and 3+) subgroups [16].

Statistical analysis

The analysis of statistical difference between cohorts of tumors and normal specimens was carried out with the non-parametric Mann-Whitney U test. The chi-square was used to analyse the potential associations between CSE1L expression status and other categorical clinicopathological variables. Survival curves were plotted by the Kaplan Meier method and compared by the log-rank test. The significance of various variables for survival was analyzed by the Cox proportional hazards model in the univariate and multivariate analysis. The level of significance was defined at a probability

Table 1. Correlation between clinical pathological factors and CSEL1 expression

Characteristics	Cases	Nuclear expression			Cytoplasmic expression		
		Low	High	*P*	Low	High	*P*
Age							
< 50 y	210	63	147	0.510	86	124	0.096
≥ 50 y	160	43	117		52	108	
Tumor							
≤ 2 cm	109	41	68	0.014	42	67	0.751
> 2 cm	261	65	196		96	165	
LNM							
Negative	160	56	104	0.018	67	93	0.112
Positive	210	50	160		71	139	
Stage							
I	48	18	30	< 0.001	22	26	0.422
II	196	69	127		71	125	
III	126	19	107		45	81	
ER							
Negative	213	62	151	0.820	80	133	0.904
Positive	157	44	113		58	99	
PR							
Negative	154	44	110	0.978	57	97	0.924
Positive	216	62	154		81	135	
HER-2							
Negative	288	81	207	0.676	110	178	0.504
Positive	82	25	57		28	54	
P53							
Negative	71	24	47	0.285	32	39	0.132
Positive	299	82	217		106	193	
Ki67							
< 10%	121	34	87	0.871	52	69	0.115
≥ 10%	249	72	177		86	163	
Subtype							
Luminal-A	61	20	41	0.842	25	36	0.239
Luminal-B	189	51	138		71	118	
Her-2(+)	84	25	59		34	50	
Triple negative	36	10	26		8	28	

value of less than 0.05 ($P < 0.05$). All statistical analyses were performed using the SPSS software (version 18).

Results

CSE1L protein overexpression in breast cancer

IHC was performed to evaluate the difference in CSE1L protein expression levels between breast cancer tissues and normal tissues. CSE1L protein expression was significantly increased in the nucleu and cytoplasm of cancer cells compared with normal tissues (**Figure 1**). We noted that 9 (11.1%) cases exhibited high nuclear CSE1L expression and that 15 (18.5%) cases exhibited high cytoplasmic CSE1L expression among 81 normal control specimens. Among 370 breast cancer specimens, we noted positive nuclear CSE1L expression in 264 (71.4%) samples ($P < 0.001$). Significantly greater cytoplasmic CSE1L expression was observed in 232 (62.7%) cancer tissues ($P < 0.001$).

Association between CSE1L protein expression and the clinicopathological variables of breast cancer

We investigated the association between CSE1L protein expression and the clinicopathological variables in breast cancer. **Table 1** showed that high nuclear CSE1L expression was significantly associated with tumor ($P = 0.014$), lymph node metastasis ($P = 0.018$) and stage ($P < 0.001$). However, cytoplasmic CSE1L expression was not associated with any clinicopathological parameters in this study.

Correlation of CSE1L protein expression with overall survival

The 5-year overall survival rate of the high nuclear CSE1L expression group was significantly worse than that of the low nuclear CSE1L group ($P = 0.011$) (**Figure 2**). Univariate Cox regression analysis showed that tumor ($P = 0.020$), lymph node metastasis ($P < 0.001$), Her2 positivity ($P = 0.012$) and CSE1L nuclear expression ($P = 0.013$) were significant predictors of poor overall survival. Furthermore, multivariate Cox regression analysis showed that lymph node metastasis ($P < 0.001$) and nuclear CSE1L expression ($P = 0.039$) were independent prognostic factors for poor overall survival in patients with breast cancer (**Table 2**).

Discussions

In this study, CSE1L was highly expressed in breast cancer tissues compared with normal

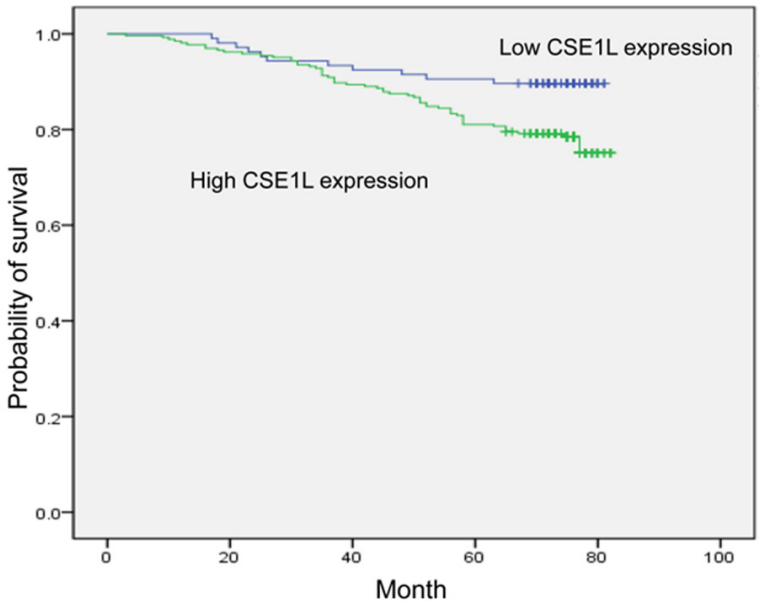

Figure 2. Kaplan-Meier survival curves stratified according to CSE1L expression in patients with breast cancer. Patients in the CSE1L high-expression group had a significantly poorer prognosis than those in the CSE1L low-expression group (Log-rank, $P = 0.011$).

tissues. Furthermore, high nuclear CSE1L expression was correlated with tumor size, lymph node metastasis, stage and adverse outcomes. There were no significant correlations between cytoplasmic CSE1L expression and clinicopathological variables.

CSE1L encodes a 971-amino acid protein with an approximate molecular weight of 100-kDa. In breast cancer, CSE1L was localized in the nucleus, cytoplasm, stroma and gland lumen [6, 17, 18]. CSE1L was overexpressed in a predominantly nuclear pattern [6], and there was a positive correlation between its expression and distant metastasis in breast cancer [16]. In addition, pathological reports have demonstrated that high CSE1L expression was correlated with the stage and grade in other cancers [4, 19], and that patients with high CSE1L levels experience worse outcomes [7, 9, 10]. Stella et al reported that serum CSE1L expression was detected in 74.5% of colorectal cancer patients with lymph node metastase, and that the ratio of positive expression increased with the increasing stage [20]. Moreover, evidence suggest that higher level of secretory CSE1L are present in the sera of patients with metastatic cancer, including colorectal, lung, breast, cervical and bile duct cancer [17].

It has been suggested that CSE1L impacts the motility and invasiveness of ovarian cancer cells [21]. Increased CSE1L expression promotes protrusion extension and migration by increasing α-tubulin and β-tubulin stability in MCF-7 cells [14]. Moreover, it was reported that CSE1L regulates MMP-2 translocation and secretion [22]. Silencing CSE-1L can inhibit B16-F10 cells metastasis to the lungs and colorectal tract in C57BL/6 Mice [20, 22]. These results indicate that CSE1L has a key role in cancer cell metastasis.

CSE1L is considered a proliferation-related protein, due to its association with microtubules and the mitotic spindles. CSE1L plays a role in promoting the mitotic phase of the cell cycle and facilitate accurate chromosomes segregation [3, 13]. CSE1L immunoreactivity has been shown to be positively correlated with proliferation markers in a variety of cancers, including lymphomas, hepatocellular carcinoma and serous ovarian carcinoma [5, 7-9]. However, enhanced CSE1L expression does not increase cancer cell proliferation [22, 23].

CSE1L is also necessary for cell apoptosis. Previous studies have shown that CSE1L promotes apoptosis induced by bacterial toxins, tumor necrosis factor [2], interferon-γ [24], doxorubicin, 5-fluorouracil, cisplatin and tamoxifen [25]. CSE1L enhances interferon-γ-induced Caspase-3 expression [24]. IHC results also indicated that CSE1L expression was correlated with Caspase-3 expression in endometrial carcinoma [12]. CSE1L regulates p53 protein accumulation induced by chemotherapeutic drugs, leading to cell apoptosis [24-26]. Activated AKT promotes the nuclear accumulation of CSE1L by phosphorylating Ran binding protein 3 [21]. CSE1L has implicated in the nuclear to cytoplasmic translocation of importin-α [27], which is necessary for the nuclear transport of p53 [28]. In addition, CSE1L can interact with a subset of p53 target gene promoters, and regulate the expression of several genes, including

Table 2. Prognostic factors in cox proportional hazards model

Variables	Univariate analysis			Multivariate analysis		
	HR	95% *CI*	*P*	*HR*	95% *CI*	*P*
Tumor size						
≤ 2 cm/> 2 cm	2.089	1.122-3.890	0.020	1.758	0.933-3.313	0.081
LNM						
Positive/Negative	3.092	1.747-5.474	< 0.001	3.605	1.719-5.465	< 0.001
ER						
Positive/Negative	0.810	0.501-1.311	0.392	1.135	0.650-1.984	0.656
PR						
Positive/Negative	0.656	0.411-1.048	0.078	0.764	0.420-1.393	0.368
HER-2						
Positive/Negative	1.901	1.154-3.131	0.012	1.704	0.969-2.994	0.064
P53						
Positive/Negative	0.777	0.445-1.358	0.377	0.768	0.428-1.376	0.374
Ki67						
Positive/Negative	1.743	0.998-3.046	0.051	1.218	0.670-2.213	0.519
CSEL1						
High/Low	2.253	1.184-4.289	0.013	1.986	1.034-3.815	0.039

anti-apoptotic and proapoptotic genes [29]. However, silencing CSE1L reduces colon cancer cell viability and increases apoptosis [10]. CSE1L also inhibits paclitaxel-induced breast cancer cells apoptosis by attenuating G2/M phase cell cycle arrest and microtubule aster formation [25]. Annalisa et al found that CSE1L protects ovarian cancer cells from death by suppressing transcription of the proapoptotic RASSF1 gene [18]. Altogether, these data indicated that CSE1L may play cell-type-specific roles in apoptosis. Breast cancer patients with high CSE1L expression levels may be more sensitive to doxorubicin and tamoxifen, and more resistant to paclitaxel. This information may be useful in choosing the most effective chemotherapeutic drugs for different patient groups.

In conclusion, CSE1L is highly expressed in breast cancer, and its expression level is correlated with metastasis, advanced stage and worse outcomes. Therefore, CSE1L may have clinical utility in breast cancer screening and diagnosis, as well as therapy, and may enable improvement in cancer chemotherapy.

Disclosure of conflict of interest

None.

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