Does CSE1L Overexpression Affect Distant Metastasis Development in Breast Cancer?

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Introduction

CSE1L (chromosome segregation 1-like) is a fragment of a gene showing 59% overall protein homology to the essential yeast chromosome segregation gene CSE1. It was first discovered in 1995 [1]. CSE1L is seen in the cytoplasm and the nucleus. Nuclear CSE1L causes selective regulation of p53 transcriptional activity, and downregulation of CSE1L expression reduces apoptosis by decreasing transcription from those p53 target promoters [2, 3]. Cytoplasmic CSE1L is related to cell mitosis through microtubules and mitotic spindles [4]. For this reason, CSE1L is claimed to play a role in cancer cell proliferation [5–7]. Overexpression of CSE1L occurs in both tumors and normal human tissues that contain actively dividing cells. Importin alpha, which is a multipurpose nuclear transport receptor and responsible for the transportation of oncogene and tumor suppressor gene products in the nucleus, is also affected by CSE1L [8]. CSE1L causes cell cycle arrest that leads to carcinogenesis by increasing importin concentrations [8]. Various recent studies found that CSE1L inhibits apoptosis, induces mitosis, and causes cancer cell migration [4, 8–10]. It was also shown that CSE1L enhances invasion and metastasis [9, 11, 12]. Higher CSE1L expression levels are correlated with increased cancer stage and poor survival [13]. However, the majority of these studies were experimental and based on either animal models or in vitro cell cultures, and experimental evidence does not always match up with the results of clinical studies. Hence, the aim of this study was to show the effect of CSE1L expression on distant metastasis in breast cancer.

Patients and Methods

A total of 71 surgically treated breast cancer patients receiving similar adjuvant therapy between January 2003 and December 2010 at Ankara Oncology Training and Research Hospital were included in this study. Patient and tumor characteristics and CSE1L status were evaluated. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded archival breast tumor tissue. The results of CSE1L staining were analyzed according to the percentage of immunoreactive cells. Results: 34 patients had distant metastasis and 37 did not. The mean age of the patients was 50.5 ± 12.1 years. Age, tumor size, and hormone receptor status were similar in patients with distant metastasis and in those without. A statistically significant relationship was found between nuclear CSE1L expression and distant metastasis of breast cancer. Lymph node metastasis and nuclear grade were other factors affecting distant metastasis. Conclusion: There is a relationship between nuclear CSE1L overexpression and distant metastasis in breast cancer. CSE1L status may therefore become a valuable prognostic tool in the future.
characteristics were recorded. The CSE1L status of the breast tumors was evalu-
ated by immunohistochemical staining, and any relationship between the re-
corded characteristics and the CSE1L status (both nuclear and cytoplasmic
CSE1L expression) was investigated. The study protocol was approved by the
Ethics Committee of Ankara Oncology Training and Research Hospital, An-
kara, Turkey. All patients included in this study were female, and all underwent
either modified radical mastectomy (MRM) or breast-conserving surgery (BCS)
performed by experienced surgeons for invasive ductal carcinoma. Male sex,
multifocal tumor, and breast cancer other than invasive ductal carcinoma were
excluded. Patients without distant metastasis were those who achieved a mini-
mum 5-year disease-free survival, and patients with distant metastasis were
those who showed systemic relapse at any time after treatment completion.
A total of 71 patients met the inclusion criteria of this study. Of those, 34 had dis-
tant metastasis and 37 achieved a minimum 5-year disease-free survival. Age,
menstrual status, tumor characteristics (size, grade, hormone receptor status),
auxillary lymph node status, type of surgery, and site of distant metastasis were
recorded by both surgeons and medical oncologists.

Immunohistochemical staining was carried out by an experienced patholo-
gist on formalin-fixed, paraffin-embedded archival primary tumor tissue of all
71 breast cancer patients to determine the CSE1L status. Staining for CSE1L
was performed on 5 μm-thick slices using the GeneTex CSE1L antibody rabbit
polyclonal antibody (1:100) (GeneTex, Inc., Irvine, CA, USA) and a Ventana
Ultraview DAB detection kit in a Ventana BenchMark XT processor (Ventana,
Tucson, AZ, USA). Antigen retrieval was a standard automated process on the
Ultraview DAB detection kit in a Ventana BenchMark XT processor (Ventana,
Tucson, AZ, USA). Antigen retrieval was a standard automated process on the
Ventana BenchMark XT at 37 °C for 16 min. The sites of peroxidase activity
were visualized using diaminobenzidine (3,3'-diaminobenzidine tetrahydro-
chloride) as substrate, and were counterstained using Mayer’s hematoxylin. The
technique and the semiquantitative immunohistochemical scoring system used
in this study were the same as in previous published studies [13, 14]. The per-
centage of immunoreactive cells was combined with the estimated staining in-
tensity. According to these 2 parameters, the tumor was scored in terms of nu-
clear or cytoplasmic staining. The staining intensity score varied from 0 to 3 (0
= no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining).
The total possible intensity score 300 was calculated using the following for-
mula: (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 negative CSE1L expression (CSE1L staining 0 and 1+) and
positive CSE1L expression (CSE1L staining 2+ and 3+) subgroups. A total of 77
immunohistochemical slices were examined using an Olympus BX51 micro-
scope (Olympus, Center Valley, PA, USA). A representative immunohisto-
chemical image of nuclear CSE1L staining is shown in figure 1.

All analyses were performed using the Statistical Package for Social Sciences
(SPSS) version 15.0 (IBM Corp., Armonk, NY, USA). Age, tumor size, estrogen
receptor (ER) status, progesterone receptor (PR) status, human epidermal growth
factor receptor 2 (HER2) status, grade, lymph node status, and cytoplasmic and
nuclear CSE1L expression were the factors analyzed for their potential effect on
distant metastasis. A p value less than 0.05 was considered statistically significant.
The Mann-Whitney U test was used to compare abnormal continuous data,

whereas the chi-square test was used to compare categorical variables. Fisher’s
exact test was used to determine whether there was a relationship between distant
metastasis and CSE1L immunohistochemical staining. The multivariate analysis
of variance (MANOVA) test was used to investigate independent factors.

Table 1. General patient characteristic and their correlation with distant
metastasis

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Metastasis (+)</th>
<th>Metastasis (–)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<tr>
<td>&lt; 50</td>
<td>23 (32)</td>
<td>12</td>
<td>11</td>
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<tr>
<td>≥ 50</td>
<td>48 (68)</td>
<td>22</td>
<td>26</td>
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<tr>
<td>Tumor size</td>
<td></td>
<td></td>
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<tr>
<td>T1</td>
<td>15 (21)</td>
<td>3</td>
<td>12</td>
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<tr>
<td>T2</td>
<td>45 (64)</td>
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<td>T3</td>
<td>11 (15)</td>
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<tr>
<td>Grade</td>
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<td></td>
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</tr>
<tr>
<td>1</td>
<td>4 (6)</td>
<td>1</td>
<td>3</td>
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<tr>
<td>2</td>
<td>34 (48)</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>33 (46)</td>
<td>20</td>
<td>13</td>
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<tr>
<td>Estrogen receptor</td>
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<tr>
<td>(+)</td>
<td>60 (85)</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>(–)</td>
<td>11 (15)</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Progesterone receptor</td>
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<td></td>
<td></td>
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<tr>
<td>(+)</td>
<td>51 (72)</td>
<td>22</td>
<td>29</td>
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<tr>
<td>(–)</td>
<td>20 (28)</td>
<td>12</td>
<td>8</td>
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<tr>
<td>HER2</td>
<td></td>
<td></td>
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<tr>
<td>(+)</td>
<td>22 (31)</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>(–)</td>
<td>49 (69)</td>
<td>25</td>
<td>24</td>
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<tr>
<td>Lymph node status</td>
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<tr>
<td>(+)</td>
<td>44 (62)</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>(–)</td>
<td>27 (38)</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Cytoplasmic staining</td>
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</tr>
<tr>
<td>(+)</td>
<td>42 (59)</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>(–)</td>
<td>29 (41)</td>
<td>12</td>
<td>17</td>
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<tr>
<td>Nuclear staining</td>
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<tr>
<td>(+)</td>
<td>61 (86)</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>(–)</td>
<td>10 (14)</td>
<td>1</td>
<td>9</td>
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</table>

Results

A total of 71 female patients who had been surgically treated for
breast cancer at our hospital were included in this study. The mean
age of the patients was 50.5 ± 12.1 years. 68% of the patients were
older than 50 years, and 57% were postmenopausal. The American
Joint Committee on Cancer (AJCC) 2010 classification was used
for staging. Accordingly, 2 patients were in stage I, 55 patients were
in stage II, and 14 patients were in stage III at the time of surgery.
MRM was the applied surgical procedure in 58 (82%) patients and
BCS in 13 (18%) patients. Distant metastasis occurred in 34 pa-
tients during the follow-up period. Age, tumor size, applied surgi-
cal procedure, menopausal status, and hormone receptor status
were similar in patients with distant metastasis and in those with-
out. Tumor and patient characteristics and their correlation with
distant metastasis are shown in table 1. 13 (38%) patients had bone
metastasis only; 8 (24%) patients had visceral metastasis only, and
13 (38%) patients had mix metastasis.

Both the cytoplasmic and the nuclear expression of CSE1L were
investigated on primary tumor tissue by immunohistochemical
staining. Nuclear CSE1L staining was observed in 33 of the 34 pa-
tients with distant metastasis while cytoplasmic CSE1L staining was
observed 22 of those 34 patients. Only 10% (n = 7) of the overall
patient population showed neither cytoplasmic nor nuclear CSE1L.
staining. Grade 1, 2, and 3 tumors showed cytoplasmic CSE1L expression levels of 75, 56, and 61%, respectively, and nuclear CSE1L expression levels of 100, 79, and 91%, respectively. There was no statistically significant correlation between cytoplasmic or nuclear CSE1L expression and age, tumor size, nuclear grade, hormone receptor status, and HER2. Similarly, cytoplasmic or nuclear CSE1L expression did not affect axillary lymph node positivity. Furthermore, there was no relationship between cytoplasmic and nuclear CSE1L expression and the number of axillary metastatic lymph nodes. Among the factors which may have had an effect on distant metastasis (age, tumor size, ER, PR, HER2, grade, lymph node status, cytoplasmic/nuclear CSE1L staining), grade (p = 0.04), lymph node status (p = 0.004), and nuclear CSE1L staining (p = 0.009) were significant in univariate analysis. In multivariate analysis of variance (MANOVA), lymph node status (p = 0.002) and nuclear CSE1L staining (p = 0.0001) were independent factors affecting distant metastasis. Nuclear CSE1L staining results in distant metastasis-negative and -positive patients are shown in figure 2. Patients with multiple metastasis tended to show higher nuclear CSE1L expression; however, the result was not significant (p = 0.06) (fig. 3).

Discussion

Breast cancer is the most common cancer type among women worldwide, and 1.67 million new cases are diagnosed each year [15]. Clinical, pathological, and biological factors such as tumor size, axillary lymph node status, hormone receptor status, and HER2 status play a role in risk stratification and affect the choice of treatment modality in breast cancer [16]. CSE1L seems to be the new biological marker for metastasis and recurrence of melanomas, lymphomas, breast cancers, hepatomas, ovarian cancer, and colon cancers, and has provided food for discussion in recent years [6, 9, 11–13, 17–19]. It was shown that CSE1L is related to microvesicle biogenesis and causes temporary extension of the plasma membrane which contributes to cancer cell migration and invasion [20]. Moreover, increased CSE1L expression enhances the secretion of matrix metalloproteinase-2 and the invasiveness of cancer cells [20]. Furthermore, reduction in CSE1L expression resulted in metastasis inhibition in B16 and F10 melanoma cells in mice [21], and overexpression of CSE1L in MCF-7 breast cancer cells increased the migration and invasiveness of cancer cells [20]. CSE1L is mapped on chromosome 20q13 which is known to harbor amplifications that correlate with aggressive breast cancer [22].

We analyzed CSE1L expression in invasive ductal carcinoma of the breast and investigated its relationship with distant metastasis. We studied both nuclear and cytoplasmic expression of CSE1L in primary tumor by immunohistochemistry and investigated potential correlations between this protein and distant metastasis of breast cancer. We also investigated the relationship between clinicopathological characteristics such as age, menopausal status, size, nuclear grade, and hormone receptor status, as well as axillary lymph node metastasis. There was no effect of both nuclear and cytoplasmic CSE1L overexpression on size, nuclear grade, and hormone receptor status of the tumor, and there was no correlation with patient age and menopausal status. Although it was claimed by experimental studies that CSE1L expression is positively correlated with high-grade cancers [6, 8, 9, 11, 18, 19, 23], we were unable to verify such a correlation. In our clinical study, we found cytoplasmic CSE1L expression percentages for grade 1, 2, and 3 tumors of 75, 56, and 61%, respectively, and the percent-
ages for nuclear CSE1L expression were 100, 79, and 91%, respectively. The small sample size of grade 1 tumors may have led to this discrepancy since only 4 patients had grade 1 tumors whereas 34 and 33 patients had grade 2 and 3 tumors, respectively. Another possible explanation for this situation is that experimental evidence does not always match up with clinical findings. In neoplastic tissues, various oncogenes are activated and various anti-oncogenes are inactivated [12, 24]. These carcinogenic processes may differ under experimental versus clinical conditions and may cause incompatibilities.

In several clinical studies, CSE1L overexpression was found to increase the invasiveness and metastatic activity of colorectal cancer cells, and is hence associated with poor patient outcome. For this reason, it may be regarded as a prognostic factor in colorectal cancer [13, 14, 17]. To the best of our knowledge, this is the first study in the literature revealing a relationship between CSE1L and distant metastasis in breast cancer. Hence, it is difficult to compare our results to previous studies. In a clinical study by Behrens et al. [9] including 50 patients and comparing benign and malignant breast tumors, invasive ductal carcinoma showed weak cytoplasmic and strong nuclear CSE1L staining while benign lesions showed predominantly weak cytoplasmic signals and slightly positive nuclear CSE1L staining. In our study, there were no benign lesions, and the breast cancer patients who presented with distant metastasis in the follow-up period showed significant nuclear CSE1L overexpression (p = 0.0001). Although the effect of cytoplasmic CSE1L on regional lymph node metastasis in colorectal cancer was revealed in an experimental study [13], we did not come to the same conclusion, probably because of the unequal number of lymph node-negative and -positive patients in the present study. Nuclear grade and axillary lymph node positivity were the other 2 factors affecting distant metastasis (p = 0.04 and p = 0.004, respectively). However, there was no correlation between nuclear CSE1L overexpression and these 2 factors.

The main finding of this study is the independent effect of nuclear CSE1L overexpression on distant metastasis in breast cancer (p = 0.0001). Nuclear CSE1L overexpression increases the rate of distant metastasis in breast cancer and consequently worsens clinical outcome, and may hence be a valuable prognostic tool.

Disclosure Statement

There is no conflict of interest for any of the authors.

References

4 Tai CJ, Shen SC, Lee WR, Liao CF, Deng WP, Chou HY, Hsieh CI, Tung JN, Chen CS, Chou JF, Li LT, Lin CY, Hsu CH, Jiang MC: Increased cellular apoptosis susceptibility (CSE1L/CAS) gene expression promotes pro-
6 Wellmann A, Krenac L, Feist T, Scher U, Pastan I, Raffeld M, Brinkmann U: Localization of the cell prolif-
7 Tai CJ, Hsu CH, Shen SC, Lee WR, Jiang MC: Cellular apoptosis susceptibility (CSE1L/CAS) protein in can-
9 Behrens P, Brinkmann U, Fogt F, Werner N, Well-