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REVIEW ARTICLE

Serum Cellular Apoptosis Susceptibility Protein for Cancer Diagnosis

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Tumor invasion and metastasis are the main cause of cancer mortality. CSE1L/CAS, the cellular apoptosis susceptibility protein, is the human homologue of the yeast chromosome segregation gene product, CSE1. Pathological studies show that CSE1L is highly expressed in various cancers, such as lung cancer, breast cancer, liver cancer, ovarian cancer, endometrial carcinomas, skin cancer, colorectal cancer, lymphomas, prostate cancers, nasopharyngeal carcinomas, medulloblastomas, and glioblastomas. The *CSE1L* gene is located on chromosome 20q13, a region that frequently harbors amplifications that correlate with cancer aggression. Experimental studies have shown that CSE1L regulates the invasion of cancer cells *in vitro* and in animal metastasis models. The results of our recent studies have revealed that CSE1L is a secretory protein present in sera from cancer patients. Significantly, there is a higher prevalence of secretory CSE1L in sera of patients with metastatic cancer. Here, we discuss the potential of CSE1L as a serum marker for the diagnosis of cancer.

1. Introduction

Cancers are known for their uncontrolled proliferation ability and they are also easy to metastasize to other tissues or other organs. Metastases are the main cause of cancer-induced mortality. Unfortunately, many malignant cancer cells that metastasize to other sites or lymph nodes surrounding the tumor are not easily detected, and the disease often recurs after the tumor has been resected. The high recurrence potential of cancer also makes it an especially distressing disease. Diagnosing metastasis is crucial for determining therapeutic strategy in the treatment of cancer. Metastatic tumors often secrete extracellular matrix (ECM)-degrading proteinases to help them invade adjacent tissue and metastasize to lymph nodes or other organs.[1] Blood proteins secreted by metastatic cancer cells should therefore be useful for screening, diagnosis, prognosis, assessment of therapeutic response, and monitoring for recurrence of cancer.[2,3] Serum CSE1L/CAS, the cellular apoptosis susceptibility protein, may be a potential marker for diagnosing cancer metastasis. The *CSE1L* gene encodes an approximately 100-kDa molecular mass protein distributed in the cytoplasm of cells.[4] CSE1L was identified in 1995 by Brinkmann et al[5] as the human homologue of the yeast chromosome segregation gene product, CSE1, that can associate with microtubules and mitotic spindles.[6] Therefore, there has been speculation that CSE1L could play a role in cancer cell proliferation.[5–11] However, there are currently no reports of experiments showing that CSE1L stimulates the proliferation of cancer cells. Our recent studies have shown that CSE1L regulates invasion and metastasis but not proliferation of cancer,[12] and that CSE1L is a secretory protein present in sera from cancer patients.[13] Here, we discuss the association of serum CSE1L with cancer and its potential for cancer diagnosis.

2. The Expression of CSE1L in Cancer

CSE1L is highly expressed in most cancers and pathological studies have shown that its expression is positively correlated with advanced cancer stage and grade. Results of immunohistochemical studies have shown that CSE1L is highly expressed in hepatocellular carcinoma compared with normal hepatocytes.[11,14] Benign breast lesions show weak CSE1L staining, whereas 70–90% of breast tumor cells showed heavy CSE1L staining.[10] Studying the expression of CSE1L in 27 benign and 55 malignant melanocytic lesions (including 32 primary and 23 metastatic lesions) revealed positive staining for CSE1L in 13 of 27 benign lesions, 5 of 7 lentigo maligna melanomas, and 11 of 12 superficial spreading melanomas. Furthermore, all acrolentiginous ($n = 7$) and nodular ($n = 6$)

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melanomas showed medium to high CSE1L staining, and all metastatic melanomas ($n = 23$) showed strong CSE1L staining.[8] Also, CSE1L staining increased from 43 ± 34% CSEL-positive cells in Stage I, to 53 ± 26% in Stage II, 68 ± 24% in Stage III, and 72 ± 24% in Stage IV.[8] Moderate to strong immunostaining of CSE1L was observed in 34 of 41 cases (83%) of serous ovarian carcinomas and was positively related to tumor grade ($p = 0.0107$) and adverse outcomes ($p = 0.0035$) of the disease.[15] A tissue array study composed of 244 serous ovarian tumors of different grades (0–3) and stages (I–IV) showed a higher expression of CSE1L in poorly differentiated invasive ovarian tumors compared with highly differentiated ones.[16] CSE1L was expressed in 93% of endometrial carcinomas, whereas lower levels of CSE1L expression were observed in the adjacent endometrium ($p = 0.003$).[17]

Papay et al[18] studied the immunophenotypic profiling of non–small-cell lung cancer progression using tissue microarray with 59 tissue samples, including 33 primary tumors without distant metastasis and 26 non–small-cell lung cancer with brain metastases and showed that elevated expression of CSE1L was significantly associated with the metastatic potential of non–small-cell lung cancer. Wellmann et al[7] showed that malignant non-Hodgkin's lymphoma and malignant cells of Hodgkin's disease both displayed very strong CSE1L staining; in contrast, normal lymphoid tissue and low-grade non-Hodgkin's lymphoma revealed weak CSE1L staining. Seiden-Long et al[19] reported that CSE1L is highly expressed in primary and metastatic human colorectal carcinoma compared with the normal colon mucosa ($p < 0.0001$). We reported recently that distribution of CSE1L in the epithelial glands of neoplastic colorectal epithelium was related with the malignance of colorectal cancer.[20] CSE1L was also found to be expressed in pilocytic astrocytomas.[21]

The results of these pathological studies show that CSE1L is highly expressed in cancer and its expression is positively correlated with advanced cancer stage and grade. Therefore, CSE1L may play an important role in the regulation of cancer progression and may serve as a diagnostic marker for cancer.

3. Mechanisms of CSE1L in Cancer Progression

CSE1L is highly expressed in cancer, where its increased expression is mainly because of amplification of the copy number of the *CSE1L* gene in cancer tissue. Chromosome 20q13 amplifications are frequently correlated with the aggression of cancer.[22–24] The *CSE1L* gene is located on chromosome 20q13 and the copy number of the *CSE1L* gene is increased in human colorectal, breast, and bladder cancer cell lines.[25] Furthermore, array-based comparative genomic hybridization studies have shown high frequency amplification of the *CSE1L* gene in glioblastoma multiforme,[26] medulloblastomas,[27] gliomas,[28] prostate cancer,[29] and nasopharyngeal carcinomas.[30] Whether other intracellular signal-transduction factors also regulate the expression or activity of CSE1L and consequently modulate cancer progression is not fully known, although we have reported previously that interferon-γ treatment can increase the level of CSE1L expression in colorectal cancer cells.[31]

CSE1L is a microtubule-associated protein that associates with microtubules and mitotic spindles.[5,6] Mitotic spindles are cellular organelles, which play a role in chromosome segregation in the cell cycle during cell division.[32] Therefore, it has been speculated that CSE1L plays a role in cancer cell proliferation.[5–11] However, the rate-limiting step in cell proliferation is mainly at the G1-S phase rather than at the mitotic phase of the cell cycle.[33,34] Also, there are currently no reports of experiments showing that CSE1L can stimulate the proliferation of cancer cells; our recent studies have shown that increased CSE1L expression in cancer cells is unable to increase the proliferation of cancer cells.[12] Our studies have further shown that CSE1L regulates invasion and metastasis of cancer cells.[12]

The mechanism through which increased CSE1L expression in cancer promotes cancer progression is becoming clearer. Microtubules are part of cell structures that play an important role in determining cell shape, which is in turn thought to be involved in cancer progression by regulating the migration of cancer cells.[35,36] Our recent study shows that the association of CSE1L with microtubules is related with protrusion extension and migration of Michigan Cancer Foundation-7 (MCF-7) cancer cells.[37] We demonstrated that increased CSE1L expression in MCF-7 breast cancer cells resulted in decreased tyrosine phosphorylation of α- and β-tubulins, increased α- and β-tubulin association, and enhanced the assembly of microtubules.[37] The formation and extension of cancer cell protrusions facilitate the migration and invasion of cancer cells to other organs;[38] increased CSE1L expression increased the protrusion extension of MCF-7 cancer cells.[37] Furthermore, our *in vitro* migration assay showed that enhanced CSE1L expression increased the migration of MCF-7 cancer cells.[37] These results indicate that CSE1L plays a role in regulating the extension of cell protrusions and the migration of cancer cells.

Matrix metalloproteinases (MMPs) are enzymes involved in the degradation of the ECM during cancer metastasis.[39] Cancer cells that develop an enhanced ability to secrete ECM-degradation proteinases are more likely to metastasize. Our other studies show that CSE1L is colocalized with MMP-2-containing vesicles, and enhanced CSE1L expression facilitates the translocation of MMP-2-containing vesicles to cell protrusions.[12,13] Furthermore, our studies show CSE1L is colocalized with MMP-2 in vesicles surrounding the outside of MCF-7 cell membranes;[13] enhanced CSE1L expression in B16-F10 melanoma cells, a highly metastatic cancer cell line, increased the secretion of MMP-2 from B16-F10 melanoma cells and enhanced cell invasion.[13] Animal tumor metastasis models have also showed that reduced CSE1L expression in B16-F10 melanoma cells decreased the pulmonary metastasis of the cells in C57BL/6 mice.[40,41] These results suggest that besides regulating the extension of cell protrusions and the migration of cancer cells through its interaction with microtubules, CSE1L may also promote the invasion of cancer cells by regulating the secretion of MMP-2-containing vesicles.

4. CSE1L in Serum From Cancer Patients and Association of Serum CSE1L With Cancer Metastasis

The colocalization of CSE1L with MMP-2 in vesicles surrounding the outside of cell membranes indicates that CSE1L is a secretory protein. Glands are the major tubular organs that mediate passage and control homeostasis by modifying secretion. Our prior immunohistochemistry study further showed CSE1L staining in the gland lumen of metastatic colorectal cancer specimens.[41] CSE1L staining in the gland lumen of cancer lesions indicates that CSE1L is secretory in cancer lesions. Figure 1 is an immunohistochemical image showing CSE1L staining in cells in tumor glands and in the gland lumen of metastatic breast cancer specimens (Figure 1). By immunoblotting with conditioned medium harvested from serum-starved B16-F10 melanoma cells, we proved that CSE1L can be secreted by B16-F10 melanoma cells.[13,41] Our recent study showed that CSE1L is also present in the cerebrospinal fluids of patients suffering from intracerebral hemorrhage caused by stroke and neurotrauma.[42] Most importantly, the results of our immunoblotting studies also showed that CSE1L is detectable in sera from cancer patients.[13,41] Serum CSE1L was detected in patients with metastatic, invasive, and primary cancers, as well as in sera from healthy donors.[13,41] However, the concentration of CSE1L in serum is positively correlated with patients' cancer stage.[13,41] And the

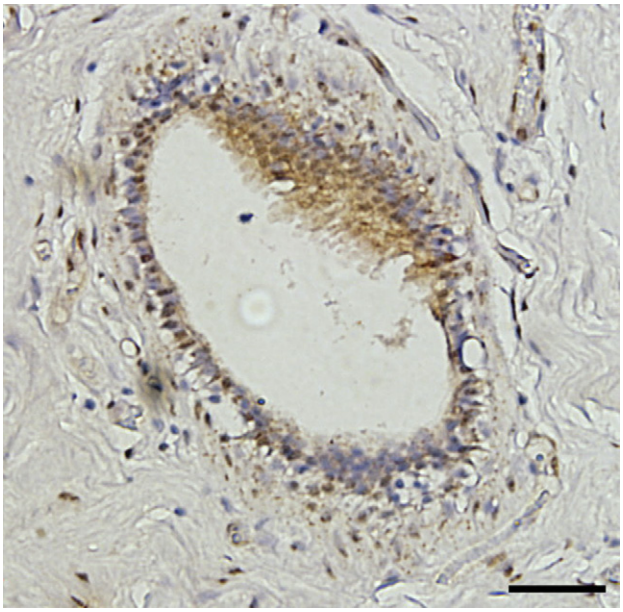

Figure 1 CSE1L distribution in breast cancer specimens analyzed by immunohistochemistry with anti-CSE1L antibodies. Note CSE1L staining in the gland lumen of the breast cancer lesion. Scale bar = 50 μm.

dose of CSE1L in sera is also higher in the sera of cancer patients than in the sera of healthy individuals.[13,41]

Colorectal cancer has a high rate of metastasis and recurrence.[43–46] We also studied the prevalence of serum CSE1L in patients with colorectal cancer, and our results showed that serum CSE1L has a close relationship to lymphatic metastasis of colorectal cancer.[41] Combining serum CSE1L and carcinoembryonic antigen assays further increase the sensitivity of metastatic colorectal cancer assays.[41] Further research is merited to see what role combined serum CSE1L and carcinoembryonic antigen assays could play in diagnosis of metastatic colorectal cancer.

5. Conclusion

The presence of secretory CSE1L in the sera of cancer patients is not restricted to a specific cancer type. Serum CSE1L has been detected in various cancer types, including lung cancer, ovarian cancer, colorectal cancer, esophageal cancer, breast cancer, cervical cancer, bile duct cancer, oviduct omental cancer, and head and neck cancer.[13,41] Therefore, CSE1L may be a serum marker that can be used for the diagnosis of most cancer types. Early detection is essential for attaining high cure rates and reducing the mortality from cancer. Screening and diagnosis of metastatic cancer are crucial for determining therapeutic strategy. CSE1L is highly present in sera of patients with metastatic cancer. Hence, CSE1L may also be a promising marker for improving the diagnostic workup of patients with metastatic cancer.

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