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Expression of CAS/CSE1L, the cellular apoptosis susceptibility protein, correlates with neoplastic progression in Barrett's esophagus

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Abstract

Background—Identifying the molecular switch responsible for the neoplastic progression of Barrett's esophagus (BE) and initiation of adenocarcinoma is clinically essential and it will have a profound impact on patient diagnosis, prognosis, and treatment. The cellular apoptosis susceptibility gene *CAS/CSE1L* is overexpressed in various cancers, including a rare report on esophageal adenocarcinoma; however, its expression in BE neoplasia has not been addressed.

Methods—We investigated the expression of the *CAS/CSE1L* protein immunohistochemically in 56 esophageal resection specimens for adenocarcinoma arising in BE. For each specimen, a full representative section of the invasive adenocarcinoma (ADC) was selected to include, when possible, BE, low grade dysplasia (LGD) and high grade dysplasia (HGD). Samples were stained for *CAS/CSE1L* expression using a rabbit polyclonal antibody recognizing the N-terminus of human *CAS/CSE1L*. Protein expression levels were measured using the Allred semiquantitative

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scoring system. The data was evaluated using chi-squared statistical analysis. Gene expression Omnibus was queried for *CAS/CSE1L* and BE neoplasia.

Results—We found minimal to absent CAS/CSE1L in all BE tissue samples; however, CAS/CSE1L was upregulated in 60% of LGD and overexpressed in HGD and ADC. The results were statistically significant ($P < 0.05$). The localization of CAS/CSE1L protein was nuclear in BE; it became nuclear and cytoplasmic in LGD and HGD, and predominantly cytoplasmic in ADC. A similar progressive increase was observed for *CAS/CSE1L* gene expression.

Conclusion—These findings show changes in CAS/CSE1L during BE progression. CAS/CSE1L may represent a potential marker for dysplasia/carcinoma.

Keywords

Barrett's esophagus; neoplasia; progression; signaling pathway; CAS/CSE1L

INTRODUCTION

Identifying the mechanisms responsible for the progression of Barrett's esophagus (BE) to adenocarcinoma is clinically and scientifically relevant. To date, the treatment of BE patients is mainly clinical and the diagnosis largely relies on endoscopic and histopathologic findings. A subset of BE patients are prone to developing low-grade dysplasia (LGD), which, with time, will progress to high-grade dysplasia (HGD) and intramucosal adenocarcinoma and eventually to invasive adenocarcinoma (ADC). However, the molecular changes responsible for this transition still remain poorly understood.

The cellular apoptosis susceptibility gene (*CAS/CSE1L*) has been reported to trigger tumor cell proliferation, apoptosis, tumor invasion, and even metastasis in a variety of human cancers [1–5]. However, its functional role in BE has not been investigated. The *CAS/CSE1L* gene encodes a multifunction protein (CAS/CSE1L), which is the human homologue of the yeast *cse1* gene. In mice, *cse1* is essential for early embryonic growth and development; when mutated, it induces death [6,7]. *CAS/CSE1L* has also been implicated in the nuclear transport of several proliferation-activating proteins, transcription factors, and oncogene and tumor suppressor gene products such as p53 [6,8]. *CAS/CSE1L* has been recognized as a cellular apoptosis susceptibility gene in colonic, ovarian, urothelial and breast cancer cells [1,2,3,5,9]. In these tumor types, *CAS/CSE1L* facilitates transformation and has an antiapoptotic role. Furthermore, its expression seems associated with an adverse patient outcome in certain tumors, such as colorectal cancer, in which, CAS/CSE1L affects cellular proliferation, apoptosis, cell-cell adhesion, and invasion, thus promoting the progression of colorectal cancer [1]. Interestingly, Jiang *et al.* have reported a close association between K-Ras mutation and CAS/CSE1L expression, providing a rational strategy for targeting CAS/CSE1L in K-Ras-mutated colorectal cancer [3]. It seems that downregulation of CAS/CSE1L blocks the cell cycle in G2/M and S phases, inhibiting cell proliferation, inducing apoptosis, and suppressing colony growth capacity by colon cancer cells [1]. Therefore, this molecule may also be relevant in BE neoplasia where alterations in cell proliferation and apoptosis seem to have great importance.

The multiple molecular pathways that interplay in the progression of BE to adenocarcinoma have been defined and include restitution, cell cycle changes, apoptosis, adhesion, DNA instability, and invasion [10]. Following mucosal injury, the mucosa is rapidly healed by restitution. During this phase, in the presence of persistent gastro-esophageal reflux, the metaplastic clones are selected and propagate. Subsequently, cell cycle checkpoint regulation is lost, with genomic instability possibly contributing to slow clonal expansion and increased cell proliferation [11–12]. At the stage of HGD inhibition of apoptosis occurs. The insurgence of ADC may be preceded by alteration in cell adhesion [13], and additional cumulative genetic errors may result in the generation of multiple clones of transformed cells, thereby expanding the population of altered cells with an angiogenic or metastatic potential.

Despite these advances in the understanding of tumor progression in BE, the expression and subcellular distribution of CAS/CSE1L in BE and BE-associated neoplasms have not yet been reported in the literature.

In this study, we investigated the cellular expression and distribution patterns of CAS/CSE1L in BE and BE neoplasia in an attempt to detect changes in its expression during the progression of non-dysplastic BE to dysplasia, from LGD to HGD and to ADC.

MATERIALS AND METHODS

We obtained esophageal resection specimens for adenocarcinoma from the Moffitt Cancer Center Pathology repository. All tumors selected had adjacent areas of BE. For each specimen, full sections were selected to include, when possible, BE with no dysplasia (BE), LGD, HGD, and (ADC. All patients provided written informed consent to have their samples included in the database. Internal Review Board approval was received for conducting this study.

Immunohistochemical evaluation of CAS/CSE1L protein expression

Overall, 56 ADC tissue samples were stained for CAS/CSE1L expression using a rabbit polyclonal antibody, recognizing the N-terminus of human CAS/CSE1L (clone E10814, dilution: 1:300; retrieval CC2Std; Spring Bioscience, Pleasanton, CA), and the Ventana automated immunostainer Discovery XT (Ventana Medical Systems, Tucson, AZ). CAS/CSE1L overexpression and knockout cell lines were used as controls of specificity. In addition, a section of hepatocellular carcinoma was used as positive control, as recommended by the manufacturer (Fig. 1D). As a negative control, we used non-immune mouse sera, omitting the CAS/CSE1L antibody during the primary antibody incubation step. CAS/CSE1L protein expression levels were measured using the Allred semiquantitative scoring system [15]. The data were evaluated by chi-squared statistical analysis. *P* values 0.05 were considered significant.

CAS/CSE1L gene expression analysis using public datasets

We analyzed CAS/CSE1L gene expression profiles using three public datasets from the Gene Expression Omnibus (GEO) (16). Two platforms were considered: the Affymetrix HG-U133A GeneChip and the Illumina Human V2 array. Using GeneAnnot (17), we found three

Affymetrix HG-U133A probesets to be of highest quality, which were used for our analysis: 201111_at, 201112_s_at, and 210766_s_at. The three datasets consisted of 1) GSE1420 (8 paired normal esophageal epithelium, BE, and esophageal adenocarcinoma) (18) run on the Affymetrix HG-U133A array; 2) GSE26886 (19 esophageal squamous epithelium from healthy patients, 20 BE, 21 esophageal adenocarcinomas, and 9 esophageal squamous cell carcinoma) (19) run on the HG-U133A array; and 3) GSE13898 (64 primary esophageal adenocarcinomas, 15 BE, and 28 surrounding normal fresh frozen tissues) (20) run on the Illumina Human V2 array. Affymetrix GeneChip data were processed using RMA (21) for normalization. GSE13898 was downloaded directly from GEO. Data were visualized using boxplots, and differences among groups of samples were identified using the Kruskal-Wallis test.

RESULTS

Clinicopathological Patient Data

The age of the patients ranged between 36 and 82 years (average, 65 years; SD, 9.3 years). Fifty two patients were male and four were female. All patients underwent Ivor-Lewis esophagogastrectomy, including a standard lymph node dissection. The size of the resected tumors ranged between 0.5 and 11.7 cm (mean, 4 cm; SD, 3 cm). There were 56 ADC arising in a background of goblet-cell intestinal metaplasia and all tumors were located in the lower one-third of the esophagus. Two representative sections for each case were studied. In 10 cases intestinal metaplasia, in 20 cases LGD, and in 36 cases HGD were present adjacent to the ADC. Three (5.4%) tumors were well differentiated, 28 (50%) moderately differentiated, and 25 (44.6%) poorly differentiated. Four (7.1%) patients had stage I, 18 (32%) stage II, 24 (43%) stage III, and 10 (18%) stage IV disease. Five patients received postoperative chemotherapy. For eight patients, radiation and chemotherapy were administered after surgical resection; only two patients received preoperative chemoradiation. Most of the selected patients did not receive neoadjuvant treatment. Currently, this is not the standard of care, however, the patients in this study were treated several years ago before the new treatment guidelines were adopted.

Immunohistochemical evaluation of CAS/CSE1L protein expression

We detected negative or low CAS/CSE1L expression in BE (immunohistochemistry scores typically were 0 or <2 in BE; Fig. 1A). CAS/CSE1L reactivity was observed in 60% (12 of 20) of LGD samples. High CAS/CSE1L expression levels were seen in HGD and ADC (immunohistochemistry scores >4; Fig. 1B and C). Using the chi-squared test, we found significant statistical differences when comparing BE vs. LGD, BE vs. HGD, BE vs. ADC; and LGD vs. HGD, and LGD vs. ADC (all P values <0.05). There was no statistical significance when comparing HGD with ADC ($P = 0.3$).

With regard to the cellular distribution of CAS/CSE1L, our results demonstrated the nuclear localization of CAS/CSE1L in BE. In samples that had progressed to LGD (Fig. 1 and 2), CAS/CSE1L protein expression remained predominantly within the nuclei, but it gradually translocated to the cytoplasm during the progression from LGD to HGD, and ADC (Figs. 2,4). Characteristically, the CAS/CSE1L nuclear stain diminished in ADC (Fig. 1C 4).

CAS/CSE1L gene expression analysis using public datasets

We queried GEO for several datasets consisting of normal esophageal epithelium, BE, and esophageal adenocarcinoma. In the GSE1420 dataset 8 paired normal epithelium, BE, and adenocarcinoma were profiled. One of three probesets (210766_s_at) demonstrated a trend toward increasing CSE1L expression from normal tissue, to BE, to adenocarcinoma ($P=0.0235$, Kruskal-Wallis test) (Fig. 4).

Interestingly, for GSE26886 dataset (19 normal squamous epithelium, 20 BE, and 21 adenocarcinomas), CAS/CSE1L expression levels were lowest in BE, with higher levels shown in adenocarcinoma and squamous cell carcinoma as well as in esophageal squamous epithelium from healthy patients ($P<6\times 10^{-7}$ and $P=0.015$).

Finally, for the GSE13898 dataset (64 adenocarcinomas, 15 BE, and 28 surrounding normal tissue), both probes demonstrated a trend of increasing CSE1L expression from LGD to HGD BE, with the highest expression levels shown in adenocarcinoma ($P=0.0494$, $P=0.0581$, Kruskal Wallis test on two probes for CAS/CSE1L).

DISCUSSION

Here we report, for the first time, changes in CAS/CSE1L protein expression levels during the progression of BE to dysplasia and invasive adenocarcinoma. Our data indicate that 1) CAS/CSE1L has a functional role early during the progression of BE to invasive adenocarcinoma and 2) the shuttling of CAS/CSE1L from the nucleus to the cytoplasm during the transition from BE to LGD and to HGD/ADC. suggests that the functional transport of this protein is deregulated during the transition from BE to ADC.

BE is diagnosed by a combination of endoscopic and pathologic findings. Histologically BE is characterized by intestinalized columnar metaplasia that extends above the lower esophageal sphincter into the tubular esophagus. BE has about 125 fold increase in risk of developing low and high grade dysplasia and to progress to ADC. CAS/CSE1L, a protein that regulates cellular proliferation and apoptosis, is synthesized in response to cellular stress and, in non-neoplastic cells, is usually localized to the nucleus [1,4,6,7,10,22,23]. CAS/CSE1L is also involved in the nuclear-cytoplasmic reshuffling of importin- α [11], in microtubule-associated cellular proliferation [12], in the migration and invasion of cancer cells [3–6,10,12–14], and in the regulation of suppressor genes, such as p53 [22]. As far as its role in nuclear-cytoplasmic reshuffling of importin- α , the CAS/CSE1L protein is actively transported into and out of the nucleus via distinct nuclear transport pathways, known as RAS-related nuclear protein (Ran). The normal nuclear localization of CAS/CSE1L is maintained by a Ran gradient [24]. Earlier studies have not clarified the impact of CAS/CSE1L cellular distribution on the carcinogenesis in the upper gastrointestinal tract, including the esophagus.

In this study, we first used public datasets from esophageal cancer gene expression profiling to demonstrate increased *CAS/CSE1L* expression as a relevant oncogenic modification during the progression of BE-associated neoplasia. Next, we correlated the CAS/CSE1L protein expression and localization patterns with the different stages of progression of

Barrett's neoplasia. Our data show that there is a stepwise upregulation of CAS/CSE1L protein expression during the progression from BE to LGD and from HGD to ADC. It is conceivable that, in ADC, the total amount of CAS/CSE1L protein remains unchanged as compared to HGD, but it is re-distributed and dissipated into the cytoplasm. This scenario has been previously depicted in other tumor types where a carcinogenic, proliferative, or anti-apoptotic role of CAS/CSE1L was proposed [4,5,22]. For example, in colonic adenocarcinoma, cytoplasmic CAS/CSE1L was reported to correlate with cancer stage, depth of tumor penetration, and lymph node metastasis [22,23]. The authors suggested that the immunohistochemical analysis of cytoplasmic CAS/CSE1L in colorectal adenocarcinoma might provide a useful aid to prognostication [23].

Therefore, it is possible that the increased cytoplasmic accumulation of CAS/CSE1L in esophageal ADC may also be utilized as a marker of BE neoplastic progression.

In conclusion, we report for the first time the expression of CAS/CSE1L in BE neoplasia and esophageal ADC. Our findings show a modification in the expression of CAS/CSE1L during the progression of BE to dysplasia and to ADC. Additional studies are warranted to prove the role of CAS/CSE1L in the progression of BE to dysplasia and cancer. The cytoplasmic localization of CAS/CSE1L seems to predict malignant transformation and may represent a prognostic marker in the management of patients with BE.

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Figure 1.

Figure 1A. A case of Barrett's esophagus without dysplasia (BE) exhibiting exclusively nuclear expression of CAS/CSE1L. Original magnification 400x.

Figure 1B. Focus of Barrett's esophagus with high grade dysplasia (HGD) exhibiting strong nuclear and cytoplasmic CAS/CSE1L expression. Original magnification 200x.

Figure 1C. Invasive adenocarcinoma (ADC) showing cytoplasmic and nuclear CAS/CSE1L expression. Original magnification 200x.

Figure 1D: A section of hepatocellular carcinoma was used as positive control for the CAS/CSE1L antibody. Original magnification 400x.

Figure 4.

CAS/CSE1L gene expression in GSE1420. CAS/CSE1L expression levels for patients with normal esophageal epithelium (N), Barrett's esophagus (B), and esophageal adenocarcinoma (ADC) were characterized using Affymetrix HG-U133A GeneChips. A significant difference in expression was found between groups ($P=0.0235$, Kruskal-Wallis test).