Promoter hypermethylation of CDH13 is a common, early event in human esophageal adenocarcinogenesis and correlates with clinical risk factors

Zhe Jin1, Yulan Cheng1, Alexandru Olaru1, Takatsugu Kan1, Jian Yang2, Bogdan Paun3, Tetsuo Ito1, James P. Hamilton1, Stefan David1, Rachana Agarwal1, Florin M. Selaru1, Fumiaki Sato1, John M. Abraham1, David G. Beer1, Yuriko Morii4, Yutaka Shimada1 and Stephen J. Meltzer1,4,6

1Division of Gastroenterology, Department of Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD
2Division of General Thoracic Surgery, Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI
3Department of Surgery, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
4Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD

Although the CDH13 gene has been shown to undergo epigenetic silencing by promoter methylation in many types of tumors, hypermethylation of this gene in Barrett’s-associated esophageal adenocarcinogenesis has not been studied. Two hundred fifty-nine human esophageal tissues were therefore examined for CDH13 promoter hypermethylation by real-time methylation-specific PCR. CDH13 hypermethylation showed discriminative receiver-operator characteristic curve profiles, sharply demarcating esophageal adenocarcinoma (EAC) from esophageal squamous cell carcinoma (ESCC) and normal esophagus (NE) \( (p < 0.0001) \). CDH13 normalized methylation values (NMV) were significantly higher in Barrett’s esophagus (BE), dysplastic BE (D) and EAC than in NE \( (p < 0.0000001) \). CDH13 hypermethylation frequency was 0% in BE, but increased early during neoplastic progression, rising to 70% in BE, 77.5% in D and 76.1% in EAC. Both CDH13 hypermethylation frequency and its mean NMV were significantly higher in BE with than without accompanying EAC. In contrast, only 5 (19.2%) of 26 ESCCs exhibited CDH13 hypermethylation. Furthermore, both CDH13 hypermethylation frequency and its mean NMV were significantly higher in EAC than in ESCC, as well as in BE or D vs. ESCC. Interestingly, mean CDH13 NMV was significantly lower in short-segment than in long-segment BE, a known clinical risk factor for neoplastic progression. Similarly, BE segment length was significantly lower in specimens with unmethylated than with methylated CDH13 promoters. 5-aza-2'-deoxycytidine treatment of OE33 EAC and KYSE220 ESCC cells reduced CDH13 methylation and increased CDH13 mRNA expression. These findings suggest that hypermethylation of CDH13 is a common, tissue-specific event in human EAC, occurs early during BE-associated neoplastic progression, and correlates with known clinical neoplastic progression risk factors.

Key words: CDH13; hypermethylation; EAC; ESCC

CDH13 (also known as H-cadherin and T-cadherin), a member of the cadherin gene superfamily, is isolated and has been mapped to 16q24, a locus that frequently undergoes deletion in human cancers, including esophageal carcinoma.\(^2,3\) In contrast to other known cadherins such as E-cadherin, N-cadherin and P-cadherin, which are transmembrane and cytoplasmic domains and is attached to the plasma membrane through a glycosyl phosphatidylinositol anchor.\(^1,4-6\) Several studies have suggested that CDH13 functions as a tumor suppressor gene and possesses potent antitumor activity in several human cancers both in vitro and in vivo.\(^7-10\) Overexpression of CDH13 in human breast carcinoma cells (MDAMB435) reduced their invasive potential in vitro and tumor formation in vivo, accompanied by reversion from invasive to normal cell morphology.\(^8\) Loss of CDH13 protein expression is associated with tumorigenicity of human non-small cell lung cancer cells.\(^8,9\) In cutaneous squamous cell carcinoma cells, overexpression of CDH13 induced a delay in the G2/M phase of the cell cycle and reduced cell proliferation.\(^10\) Downregulation of CDH13 expression has been reported in various human cancers, including those arising in the breast, lung, ovary, stomach and colon.\(^8,9,11-13\) It is now well-established that promoter hypermethylation correlates with silencing of gene transcription in many cancers,\(^14-18\) including ESCC and EAC.\(^16-18\) Furthermore, there is a growing body of evidence showing that abnormal methylation of DNA can be an early event in carcinogenesis and can serve as an early cancer detection biomarker.\(^19\) Including in EAC.\(^17-20\) Hypermethylation of CDH13 has been described in many human cancers,\(^8,14,21-25\) including ESCC\(^26,29\); however, hypermethylation of CDH13 in precancerous lesions such as Barrett’s metaplasia (BE), as well as in BE-associated EAC, is an area that still remains to be explored. We investigated hypermethylation of the CDH13 promoter by real-time quantitative methylation-specific PCR (qMSP) in 259 endoscopic esophageal biopsy specimens of differing histologies and correlated these data with clinicopathological features. Our results reveal that promoter hypermethylation of CDH13 is a common event in EAC but not in ESCC and occurs early during BE-associated esophageal neoplastic progression, correlating with clinical criteria associated with neoplastic progression risk.

Material and methods

Tissue samples

The 259 specimens examined in the current study comprised 66 from normal esophagus (NE), 60 of non-dysplastic Barrett’s metaplasia (BE, including 36 obtained from patients with BE alone (Ba) and 24 from patients with BE accompanied by EAC (Bt)), 40 from dysplastic BE [D, including 19 low-grade (LGD) and 21 high-grade (HGD)], 67 EACs and 26 ESCCs. All patients provided prior written informed consent under a protocol approved by the Institutional Review Boards at the University of Maryland School of Medicine, the Baltimore Veterans Affairs Medical Center and the Johns Hopkins University School of Medicine. Biopsies were obtained using a standardized biopsy protocol as previously described.\(^20\) Research tissues were taken from grossly apparent BE epithelium or from mass lesions in patients manifesting these changes at endoscopic examination, and histology was confirmed using parallel aliquots culled from identical locations at

Abbreviations: AUROC, area under the ROC curve; Ba, BE from Barrett’s patient; BE, Barrett’s metaplasia; Bt, BE from EAC patient; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; HGD, high-grade dysplasia in BE; LGD, low-grade dysplasia in BE; LSEB, long-segment BE; MSP, methylation-specific PCR; NE, normal esophageal epithelium; NMV, normalized methylation value; SSBE, short-segment BE; 5-Aza-C, 5-aza-2'-deoxycytidine; ROC curve, receiver-operator characteristic curve; RT-PCR, reverse-transcription polymerase chain reaction.

Grant sponsor: NIH; Grant numbers: CA085069, CA084986, CA108603, CA106765.

*Correspondence to: Division of Gastroenterology, Department of Medicine, Johns Hopkins University School of Medicine, 1503 E. Jefferson Street, Rm. 112, Baltimore, MD 21231, USA. Fax: +1-410-502-1329. E-mail: smeltzer@jhmi.edu.

Received 14 March 2008; Accepted after revision 16 June 2008 DOI 10.1002/ijc.23804

Published online 26 August 2008 in Wiley InterScience (www.interscience.wiley.com).
endoscopy. All research biopsy specimens were stored in liquid nitrogen prior to DNA extraction. Clinico-pathologic characteristics are summarized in Table I.

**Cell lines**

OE33 EAC and KYSE220 ESCC cells were cultured in 47.5% RPMI 1640, 47.5% F-12 supplemented with 5% fetal bovine serum.

**DNA and RNA extraction**

Genomic DNA was extracted from biopsies and cultured cells using a DNAeasy Tissue Kit (Qiagen, Valencia, CA). Total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA). DNAs and RNAs were stored at −80°C prior to analysis.

**Bisulfite treatment and real-time methylation-specific PCR**

One microgram DNA was treated with bisulfite to convert unmethylated cytosines to uracils prior to MSP using an Epitect Bisulfite Kit (Qiagen, Valencia, CA). Promoter methylation levels of CDH13 were determined by real-time quantitative MSP with an ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and probes for CDH13 were the same as previously reported. A standard curve was generated for normalization of data. Primers and probe for CDH13 correspond to amplified target gene mRNA expression (derived from the standard curve) in sample and control RNAs, respectively, while ACTB-S and ACTB-FM correspond to β-actin in sample and fully methylated RNAs, respectively.

**Real-time quantitative RT-PCR**

To determine CDH13 mRNA levels, one-step real-time quantitative RT-PCR was performed using a Qiagen QuantiTect Probe RT-PCR Kit (Qiagen, Hilden, Germany) and an ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and probe for CDH13 were as follows: CDH13-forward: 5'-TGTGGGAAGTTGGTTGGTG-3'; CDH13-reverse: 5'-CCCACTCCCGCGCCCTC-3'; β-actin was used for normalization of data. Primers and probe for β-actin were the same as previously reported. A standard curve was generated using serial dilutions of qPCR Reference Total RNA (Clontech, Mountainview, CA). Normalized mRNA value (NRV) was calculated according to the following formula for relative expression of target mRNA: NRV = (TarS/TarC)/(ACTB-S/ACTB-C), where TarS and TarC represent levels of target gene mRNA expression derived from the standard curve in sample and control RNAs, respectively, while ACTB-S and ACTB-C correspond to amplified ACTB levels in sample and control RNAs, respectively.

**5-Aza-dC treatment of esophageal cancer cell lines**

To determine whether CDH13 inactivation was due to promoter hypermethylation in esophageal cancer, 2 esophageal cancer cell lines (KYSE220 and OE33) were subjected to 5-Aza-dC (Sigma, St. Louis, MO) treatment as previously described. Briefly, 1 × 10^5 cells/ml were seeded onto a 100-mm dish and grown for 24 hr. Then, 1 μl of 5 mM 5-Aza-dC per ml of cells was added every 24 hr for 4 days. DNAs and RNAs were harvested on day 4.

**Table I** - Clinico-pathologic characteristics and methylation status of CDH13 in human esophageal tissues

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Number of samples</th>
<th>Age (year) mean</th>
<th>NMV</th>
<th>Methylation Status (cutoff 0.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal esophagus</td>
<td>66</td>
<td>64.3</td>
<td>0.054</td>
<td>0%</td>
</tr>
<tr>
<td>BE</td>
<td>60</td>
<td>63.7</td>
<td>0.312</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>Ba</td>
<td>35</td>
<td>62.5</td>
<td>0.262</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bt</td>
<td>24</td>
<td>65.5</td>
<td>0.387</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dysplasia in Barrett’s esophagus</td>
<td>40</td>
<td>65.3</td>
<td>0.383</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>Low-grade dysplasia</td>
<td>19</td>
<td>65.3</td>
<td>0.283</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>High-grade dysplasia</td>
<td>21</td>
<td>65.2</td>
<td>0.382</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>EAC</td>
<td>67</td>
<td>65.1</td>
<td>0.2392</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>ESCC</td>
<td>26</td>
<td>62.5</td>
<td>0.0458</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Barrett’s segment of Ba</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-segment (&lt;3 cm)</td>
<td>14</td>
<td>62.3</td>
<td>0.131</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Long-segment (&gt;= 3 cm)</td>
<td>16</td>
<td>62.8</td>
<td>0.4071</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Stage of EAC patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>63</td>
<td>0.308</td>
<td>*NS</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>65.2</td>
<td>0.2408</td>
<td>73.3%</td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>64.6</td>
<td>0.2111</td>
<td>72%</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>66.3</td>
<td>0.2921</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Lymph node metastasis in EAC patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>64.9</td>
<td>0.2751</td>
<td>75%</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>64.6</td>
<td>0.2277</td>
<td>76%</td>
</tr>
<tr>
<td><strong>Smoking status of EAC patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>6</td>
<td>58.5</td>
<td>0.2984</td>
<td>*NS</td>
</tr>
<tr>
<td>Former</td>
<td>24</td>
<td>68.5</td>
<td>0.2143</td>
<td>79.2%</td>
</tr>
<tr>
<td>Current</td>
<td>13</td>
<td>60.8</td>
<td>0.2561</td>
<td>76.9%</td>
</tr>
<tr>
<td><strong>Alcohol drinking status of EAC patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>16</td>
<td>65.3</td>
<td>0.2209</td>
<td>*NS</td>
</tr>
<tr>
<td>Former</td>
<td>15</td>
<td>63</td>
<td>0.2524</td>
<td>86.7%</td>
</tr>
<tr>
<td>Current</td>
<td>10</td>
<td>65.7</td>
<td>0.2427</td>
<td>80%</td>
</tr>
</tbody>
</table>

1BE, Barrett’s metaplasia; Ba, BE from patients with Barrett’s alone; Bt, BE from patients with Barrett’s accompanied by EA; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma. *Mann-Whitney U test; *comparisons made to normal esophagus; comparisons made to ESCC; Kruskal-Wallis test. UM, unmethylated; M, methylated; Fisher’s exact test; Chi-square for Independence test.

NS, not significant.
Data analysis and statistics

Receiver-operator characteristic (ROC) curve analysis was performed using NMVs for the 67 EAC, 26 ESCC and 66 NE specimens by Analyse-it® software (Version 1.71, Analyse-it Software, Leeds, UK). Using this approach, the area under the ROC curve (AUROC) identified optimal sensitivity and specificity levels at which to distinguish normal from malignant esophageal tissues (NE vs. EAC), yielding a corresponding NMV threshold with which to dichotomize the methylation status of CDH13. The threshold NMV value determined from this ROC curve was applied to determine the status of CDH13 methylation in all tissue types included in the study. For all other statistical tests, Statistica (version 6.1; StatSoft, Tulsa, OK) was employed. Differences with \( p < 0.05 \) were considered significant.

Results

CDH13 promoter hypermethylation in esophageal tissues

Promoter hypermethylation of CDH13 was analyzed in 66 NE, 60 BE (including 36 Ba and 24 Bt), 40 D (including 19 LGD and 21 HGD), 67 EAC, and 26 ESCC. CDH13 promoter hypermethylation showed highly discriminative ROC curve profiles and AUROCs, clearly distinguishing both EAC and ESCC from NE (Figs. 1a and 1b), as well as EAC from ESCC (Fig. 1c).

The cutoff NMV for CDH13 (0.06) was identified from the ROC curve (EAC vs. NE) to achieve the highest possible sensitivity while maintaining 100% specificity. Mean NMV and frequency of CDH13 hypermethylation for each tissue type are shown in Table I. NMVs of CDH13 were significantly higher in EAC, D, HGD, LGD, BE, Ba and Bt than in NE (\( p < 0.001, \) Mann–Whitney U test). The frequency of CDH13 hypermethylation was significantly higher in BE (70%), D (77.5%) and EAC (76.1%) than in N (0%; \( p < 0.0001, \) \( p < 0.0001 \) and \( p < 0.0001 \), respectively; Fisher’s exact test). Interestingly, both CDH13 hypermethylation frequency and mean NMV were significantly higher in Bt than in Ba (87.5% vs. 58.3%, \( p = 0.021 \) and 0.3871 vs. 0.2623, \( p = 0.045 \), respectively). The mean CDH13 NMV in EAC (0.2722) was significantly higher than that in matching NE (0.0034) for 27 cases in which matching NE and EAC were available (\( p < 0.00001, \) Wilcoxon matched pairs test). In contrast to EAC, only five (19.2%) of 26 ESCCs manifested hypermethylation of CDH13. There was no significant difference in mean CDH13 NMV between tumor and normal tissue in 13 cases for which matching ESCC (0.0337) and NE (0.0131; \( p = 0.6 \), Wilcoxon matched pairs test) were available. Both CDH13 hypermethylation frequency and mean NMV were significantly higher in EAC than in ESCC (76.1% vs. 19.2%, \( p < 0.0001 \) and 0.2392 vs. 0.0458, \( p < 0.0001 \), respectively), as well as in D vs. ESCC (77.5% vs. 19.2%, \( p < 0.0001 \) and 0.3383 vs. 0.0458, \( p < 0.0001 \), respectively) and in BE vs. ESCC (70% vs. 19.2%, \( p < 0.0001 \) and 0.3122 vs. 0.0458, \( p < 0.0001 \); Table I).

According to generally accepted criteria, BE was defined as long-segment (LSBE) if it was equal to or greater than 3 cm in length, or short-segment (SSBE) if less than 3 cm. The mean NMV of CDH13 was significantly higher in LSBE than in SSBE (0.4071 vs. 0.131; \( p < 0.01 \), Student’s t-test, Table I and Fig. 2a). Similarly, segment lengths of BEs with methylated CDH13 promoters (mean = 5.83 cm) were significantly longer than segment lengths of BEs with unmethylated CDH13 promoters (mean = 1.83 cm; \( p < 0.001 \), Student’s t-test; Fig. 2b), and the frequency of CDH13 hypermethylation frequency was significantly higher in LSBE than in SSBE (87.5% vs. 28.6%; \( p < 0.01 \), Fisher’s exact test; Table I).

No significant associations were observed between CDH13 promoter hypermethylation and patient age (data not shown), survival (log-rank test, data not shown), tumor stage, lymph node metastasis, smoking or alcohol consumption (Table I).
CDH13 methylation and mRNA levels in esophageal cancer cell lines pre- and post-5-Aza-dC treatment

KYSE220 ESCC and OE33 EAC cells were subjected to 5-Aza-dC treatment. After 5-Aza-dC treatment, the NMV of CDH13 was diminished and the mRNA level of CDH13 was increased in both KYSE220 and OE33 cells (Fig. 3).

Discussion

In the current study, we systematically investigated hypermethylation of the CDH13 gene promoter in cell lines and primary human esophageal lesions of contrasting histological types and grades by qMSP. Our results demonstrate that CDH13 promoter hypermethylation occurs frequently in human EAC, but not in ESCC. In addition, our data show that CDH13 hypermethylation increases early during esophageal adenocarcinogenesis, from 0% in NE to 58.3% in BE, 77.5% in D and 76.1% in EAC. These results imply that hypermethylation of CDH13 occurs early in most subjects, that its frequency increases during adenocarcinogenesis, and that it is tissue-specific (i.e., common in EAC but rare in ESCC). Further evidence supporting this tissue specificity is provided by ROC curves, which clearly distinguished EAC from ESCC. Similarly, support for tissue specificity is evident from the finding that both CDH13 hypermethylation frequency and mean CDH13 NMV were significantly higher in EAC than in ESCC. In addition, the low frequency (19.2%) of CDH13 hypermethylation in ESCC, as determined in the current study, is consistent with previous findings by other groups.24,29 Thus, CDH13 hypermethylation appears to constitute a critical event in only one of the two esophageal cancer subtypes.

Several studies have suggested that methylation of certain genes may occur as a field change and may be associated with an increased risk of malignant progression.17,19,20,30,35 CDKN2A, ESR1 and MYOD1 were methylated only in BE from patients who possessed dysplasia or cancer in other regions of their esophagus, but not in patients with no evidence of progression beyond BE, while CALCA, MGMT and TIMP3 were methylated more frequently in normal stomach, normal esophageal mucosa and intestinal metaplasia from patients with distant dysplasia or esophageal cancer than from patients without dysplasia or cancer.35 Previously, we demonstrated that hypermethylation of p16, RUNX3 and HPP1 in BE or LGD may represent independent risk factors for the progression of BE to HGD or EAC.30 Recently, we also found that both hypermethylation frequency and NMV of the nel-like 1, tachykinin-1, somatostatin and AKAP12 genes were higher in BE with accompanying EAC than in BE without accompanying EAC.17–20 Interestingly, both CDH13 hypermethylation frequency and level were significantly higher in BE with than without accompanying EAC in the current study, suggesting that CDH13 is a biomarker of more ominous disease lurking nearby.

In this study, we also correlated CDH13 methylation with clinicopathologic features. Despite some degree of controversy regarding the length of the BE segment as a predictive factor in BE progression, it is likely that this clinical parameter is an important predictor of neoplastic progression. In the Seattle Barrett’s Esophagus Project, BE segment length was not related to cancer risk in a prospective cohort study of 309 Barrett’s patients (p > 0.2); however, when patients with HGD at entrance were excluded, a strong trend was observed, with a 5 cm difference in length associated
with a 1.7-fold increase in cancer risk (95% CI, 0.8–3.8-fold). Significant differences in the frequency of both dysplasia and EAC were observed between SSBE and LSBE, at 81% vs 24.4% for dysplasia (p < 0.0001) and 0% vs 15.4% for EAC (p < 0.0005). In a comprehensive prospective study of 889 consecutive patients, the prevalence of dysplasia and cancer differed significantly in patients with SSBE vs LSBE. More recently, a significantly increased risk of progression to HGD or EAC with LSBE after a mean follow-up of 12.7 years was reported. In our previous studies, the *nел-1*, *tachykinin-1*, *somatostatin* and *AKAP12* genes were significantly more hypermethylated in LSBE than in SSBE. Similarly, the length of the BE segment was significantly higher in specimens with methylated than with unmethylated *CDH13* promoters. Thus, *CDH13* hypermethylation also showed a strong relationship to BE segment length. The mean NMV of *CDH13* was significantly higher in LSBE than in SSBE. Similarly, the length of the BE segment was significantly greater in specimens with methylated than with unmethylated *CDH13* promoters. Thus, *CDH13* hypermethylation may constitute a molecular correlate of BE segment length, as well as a harbinger of nearby neoplastic disease. These results also suggest that epigenetic alterations, which may account for some of the biologic behavior of BE, clearly differ between LSBE and SSBE, suggesting a need for further large-scale studies.

In the current study, the mean NMV in dysplasia was higher than in EAC. This finding could have resulted from either differences in sample sizes between these two groups, or from differential contamination by nonneoplastic cells.

In accordance with previous findings in other primary cancer cell types, we observed that methylation of *CDH13* in EAC and ESCC cancer cell lines was associated with silenced or reduced expression of *CDH13* mRNA. Treatment with 5-Aza-dC restored mRNA expression and reversed *CDH13* methylation in these cells. Restoration of *CDH13* mRNA expression by demethylating agent treatment implies that DNA hypermethylation was responsible for silencing of *CDH13*.

In summary, findings of the current study suggest that hypermethylation of the *CDH13* promoter is a common event in human esophageal adenocarcinogenesis, occurs early during Barrett’s-associated esophageal carcinogenesis, and is associated with clinical risk factors of progression. In addition, *CDH13* hypermethylation is uncommon in human ESCC, thus making it a potential cell type-specific biomarker for EAC. Further large-scale prospective longitudinal validation studies of this alteration as a predictive biomarker for EAC development are warranted by these data.

References


