Abstract

Purpose: Our aim was to investigate whether and at what stage hypermethylation of the tachykinin-1 (TAC1) gene is associated with human esophageal neoplastic transformation.

Experimental Design: TAC1 promoter hypermethylation was examined by real-time methylation-specific PCR in 258 human esophageal specimens and 126 plasma samples from patients or tissues at various stages of neoplastic evolution.

Results: TAC1 hypermethylation in tissue samples showed highly discriminative receiver-operator characteristic curve profiles, clearly distinguishing esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) from normal esophagus ($P < 0.0001$). Both frequencies and normalized methylation values of TAC1 tissue methylation were significantly higher in Barrett’s metaplasia (BE), dysplastic Barrett’s esophagus, EAC, and ESCC than in normal esophagus ($P < 0.01$). The frequency of TAC1 hypermethylation increased dramatically and early during neoplastic progression, from 7.5% in normal esophagus to 55.6% in BE from patients with Barrett’s metaplasia alone, 57.5% in dysplastic Barrett’s esophagus, and 61.2% in EAC. There was a significant relationship between TAC1 hypermethylation and BE segment length, a known clinical risk factor for neoplastic progression. Twelve (50%) of 24 ESCC exhibited TAC1 hypermethylation. Overall patient survival correlated significantly with TAC1 methylation status in ESCC patients (mean survival, 22 versus 110 months; $P = 0.0102$, log-rank test), but not in EAC patients. Both mean normalized methylation values and frequency of TAC1 hypermethylation in plasma samples were significantly higher in EAC patients than in control subjects. Treatment of KYSE220 ESCC and BICEAC cells with 5-aza-2’-deoxycytidine reduced TAC1 methylation and increased TAC1 mRNA expression.

Conclusions: TAC1 promoter hypermethylation is a common event in both major histologic types of human esophageal carcinoma, occurs early, correlates with other progression risk factors in esophageal adenocarcinogenesis, and is a tissue biomarker of a poor prognosis in ESCC. Circulating methylated TAC1 promoter DNA also offers potential as a biomarker for the diagnosis of EAC.

The tachykinin-1 (TAC1) gene has been mapped to chromosome 7q21-22 (1), a locus that frequently undergoes loss of heterozygosity in human cancers, including esophageal adenocarcinoma (EAC; refs. 2, 3). However, loss of heterozygosity is only one mechanism of gene inactivation; other common mechanisms include point mutation, homozygous deletion, and promoter methylation (4). It is now well established that promoter methylation correlates with transcriptional silencing in cancers (5), including esophageal squamous cell cancer (ESCC) and EAC (6, 7). Recently, data from our laboratory showed that the TAC1 promoter was methylated in 16 (47%) of 34 human colon cancers, and that the demethylating agent 5-aza-2’-deoxycytidine (5-Aza-dC) reversed TAC1 promoter hypermethylation and restored TAC1 mRNA expression in colon cancer cell lines (8).
This malignancy exists in two principal forms, each possessing distinct pathologic characteristics: ESCC, which occurs at high frequencies in many developing countries, especially in Asia; and EAC, which is more prevalent in Western countries, with a rapid rate of increase in recent years. Although significant advances have been made in the treatment of esophageal cancers, these aggressive malignancies commonly present as locally advanced disease, with a very poor prognosis (5-year survival; ref. 10). Therefore, to improve outcome, it is important to discover novel early detection biomarkers and new targets for chemoprevention and therapy.

There is a growing body of evidence showing that abnormal methylation of DNA is an early event in carcinogenesis and can serve as an early cancer detection or progression biomarker. Based on these findings, we hypothesized that TAC1 expression was silenced via promoter hypermethylation in human esophageal cancers, that this was an early event in the genesis of EAC, and that TAC1 hypermethylation could serve as a potential early detection biomarker for EAC.

To test these hypotheses, we studied methylation of the TAC1 gene promoter by real-time quantitative methylation-specific PCR in 258 human endoscopic esophageal specimens and 126 plasma samples from patients or tissues at various stages of neoplastic evolution. The effect of a DN methyltransferase inhibitor, 5-Aza-dC, on the reexpression of natively methylated and epigenetically silenced TAC1 was also studied in esophageal cancer cell lines. Our results show that TAC1 is silenced by promoter hypermethylation, and that this hypermethylation is a common event in both major histologic types of human esophageal carcinoma, occurs early during Barrett’s-associated esophageal neoplastic progression, and is significantly associated with a poor prognosis in ESCC patients. In addition, our data show that circulating hypermethylated TAC1 DNA is associated with the presence of EAC.

### Table 1. Clinicopathologic characteristics and methylation status of TAC1 in human esophageal tissues

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>No. samples</th>
<th>Age (y), mean</th>
<th>NMV Mean</th>
<th>Frequency (%)</th>
<th>Methylation status (cutoff 0.12)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UM</td>
<td>M</td>
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<tr>
<td><strong>Barrett’s segment</strong></td>
<td></td>
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<tr>
<td>SSBE (&lt;3 cm)</td>
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<td>0.1186</td>
<td>&lt;0.05</td>
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<tr>
<td>LSBE (≥3 cm)</td>
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<td>62.8</td>
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<tr>
<td>Normal esophagus</td>
<td>67</td>
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<td>60</td>
<td>63.7</td>
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<td>&lt;0.05</td>
<td>63.3</td>
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<tr>
<td>Ba</td>
<td>36</td>
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<td>0.2145</td>
<td>&lt;0.05</td>
<td>55.6</td>
</tr>
<tr>
<td>Bt</td>
<td>24</td>
<td>65.5</td>
<td>0.2313</td>
<td>&lt;0.05</td>
<td>75.0</td>
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<td>Dysplasia in Barrett’s esophagus</td>
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<td>65.3</td>
<td>0.1967</td>
<td>&lt;0.05</td>
<td>57.5</td>
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<tr>
<td>Low-grade dysplasia</td>
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<td>65.3</td>
<td>0.2361</td>
<td>&lt;0.05</td>
<td>63.2</td>
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<td>High-grade dysplasia</td>
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<td>65.2</td>
<td>0.161</td>
<td>&lt;0.05</td>
<td>52.4</td>
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<td>EAC</td>
<td>67</td>
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<td>0.227</td>
<td>&lt;0.05</td>
<td>61.2</td>
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<tr>
<td>Well differentiation</td>
<td>10</td>
<td>66.2</td>
<td>0.2625</td>
<td>&lt;0.05</td>
<td>60.0</td>
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<tr>
<td>Moderate differentiation</td>
<td>24</td>
<td>66.1</td>
<td>0.262</td>
<td>&lt;0.05</td>
<td>75.0</td>
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<tr>
<td>Poor differentiation</td>
<td>22</td>
<td>65.5</td>
<td>0.211</td>
<td>&lt;0.05</td>
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<td>Unknown</td>
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<td>61</td>
<td>0.1505</td>
<td>&lt;0.05</td>
<td>54.5</td>
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<td>ESCC</td>
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<td>0.1896</td>
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<td><strong>Stage of EAC patients</strong></td>
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<td>7</td>
<td>63</td>
<td>0.3038</td>
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<td>71.4</td>
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<tr>
<td>II</td>
<td>15</td>
<td>65.2</td>
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<td>53.3</td>
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<td>III</td>
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<tr>
<td>Negative</td>
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<td>64.9</td>
<td>0.2451</td>
<td>&gt;0.05</td>
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<td>64.6</td>
<td>0.1957</td>
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<tr>
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<tr>
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<td>10</td>
<td>65.7</td>
<td>0.2542</td>
<td></td>
<td>60.0</td>
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</tbody>
</table>

Abbreviations: UM, unmethylated; M, methylated.

* Student’s t test.
† Fisher’s exact test.
‡ Comparisons made with normal esophagus.
§ 2 for independence test.

(9). This malignancy exists in two principal forms, each possessing distinct pathologic characteristics: ESCC, which occurs at high frequencies in many developing countries, especially in Asia; and EAC, which is more prevalent in Western countries, with a rapid rate of increase in recent years. Although significant advances have been made in the treatment of esophageal cancers, these aggressive malignancies commonly present as locally advanced disease, with a very poor prognosis (~14% 5-year survival; ref. 10). Therefore, to improve outcome, it is important to discover novel early detection biomarkers and new targets for chemoprevention and therapy. There is a growing body of evidence showing that abnormal methylation of DNA is an early event in carcinogenesis and can serve as an early cancer detection or progression biomarker. Based on these findings, we hypothesized that TAC1 expression was silenced via promoter hypermethylation in human esophageal cancers, that this was an early event in the genesis of EAC, and that TAC1 hypermethylation could serve as a potential early detection biomarker for EAC.
Tissue and plasma samples. In the current study, endoscopic biopsies of 67 normal esophagi, 60 Barrett’s metaplasias without dysplasia [BE, including 36 obtained from patients with Barrett’s only (Ba) and 24 from patients with Barrett’s accompanied by EAC (Bt)], 40 dysplasias occurring in BE (including 19 low grade and 21 high grade), 67 EAC, and 24 ESCC were obtained from 195 patients. All normal esophageal specimens were located at least 7 cm away from any BE or other esophageal pathology. In addition, plasma samples were obtained from 35 age- and gender-matched noncancer/non-Barrett’s control subjects, 10 additional patients with Ba, 20 with dysplastic Barrett’s esophagus, and 61 with EAC. Among these specimens, matched biopsy and plasma samples were available from 17 control subjects, 3 patients with Ba, 8 with dysplastic Barrett’s esophagus (five low grade and three high grade), and 32 with EAC. EAC was staged according to the sixth edition of the tumor-node-metastasis staging system (11). All patients provided written informed consent under a protocol approved by the institutional review boards at the University of Maryland and Baltimore Veterans Affairs Medical Centers, where all esophagogastroduodenoscopies were done. Biopsies were obtained using a standardized biopsy protocol, as previously described (7).

Control subject 35 65 0.0336 8.6 32 3 <0.05*
BE 10 61 0.0046 <0.05 † 0.0 10 0
Dysplastic Barrett’s esophagus 20 64.5 0.0018 <0.01 † 0.0 10 0 <0.01 †
Low-grade dysplasia 10 62.4 0.0016 <0.01 † 0.0 10 0
High-grade dysplasia 10 66.6 0.002 <0.01 † 0.0 10 0
EAC 61 64.2 0.2237 <0.05 † 29.5 43 18

Stage of EAC patients
I 3 65.3 0.6469 >0.05 || 33.3 2 1
II 7 60.7 0.5139 57.1 3 4
III 15 64.4 0.1595 26.7 11 4
IV 7 67.1 0.3584 42.9 4 3

Lymph node metastasis in EAC patients
Negative 11 63.7 0.4449 >0.05 † 56.0 6 5 >0.05*
Positive 15 65.1 0.1596 56.0 11 4

Mean \( P \) Methylation status (cutoff 0.12)

Frequency (%) UM M P

5-Aza-dC treatment of esophageal cancer cell lines. To determine whether TAC1 inactivation was due to promoter hypermethylation in esophageal cancer, two esophageal cancer cell lines (KYSE220 and BIC) were subjected to 5-Aza-dC (Sigma) treatment as previously described (13, 14). Briefly, \( 1 \times 10^6 \) cells/mL were seeded onto a 100-mm dish and grown for 24 h. Then, 1 \( \mu \)L of 5 mmol/L 5-Aza-dC per milliliter of cells was added every 24 h for 6 days. DNAs and RNAs were harvested on day 6.

Data analysis and statistics. Receiver-operator characteristic (ROC) curve analysis (15) was done using NMs for the 67 EAC, 24 ESCC, and 67 normal esophagi by Analyse-it software (version 1.7.1, Analyse-it Research).
Using this approach, the area under the ROC curve identified optimal sensitivity and specificity levels at which to distinguish normal from malignant esophageal tissues, and corresponding NMV thresholds were calculated for TAC1. The cutoff value determined from this ROC curve was applied to determine the frequency of TAC1 methylation in each tissue type included in the present study. For all other tests, Statistica (version 6.1; StatSoft) was used. Differences with $P < 0.05$ were deemed significant.

**Results**

**TAC1 promoter hypermethylation in different esophageal tissues.** TAC1 promoter hypermethylation was analyzed in 24 ESCC and 67 EAC, 40 dysplastic Barrett’s esophagus (19 low grade and 21 high grade), 60 BE (36 Ba and 24 Bt), and 67 normal esophagi. TAC1 promoter hypermethylation showed highly discriminative ROC curve profiles, which clearly distinguished both ESCC ($P < 0.00001$) and EAC ($P < 0.00001$) from normal esophagus. ROC curves with corresponding area under the ROC curves for TAC1 of ESCC versus normal esophagus, EAC versus normal esophagus, and both types of esophageal tumors versus normal esophagus are shown in Fig. 1.

The tissue cutoff NMV for TAC1 (0.12) was chosen from the ROC curve to achieve a high sensitivity while still keeping specificity above 90%. Mean NMV and frequency of TAC1 hypermethylation for each tissue type are shown in Table 1. The NMV of TAC1 was significantly higher in ESCC, EAC, dysplastic Barrett’s esophagus (high grade and low grade), Ba, Bt, and BE than in normal esophagus ($P < 0.000001$, Student’s $t$ test). Similarly, increased frequencies of TAC1 hypermethylation were observed in Ba (55.6%), dysplastic Barrett’s esophagus (57.5%), and EAC (61.2%) relative to normal esophagus (7.5%), yielding a sensitivity of 61.2% (41 of 67) and a specificity of 92.5% (62 of 67) for the diagnosis of EAC. Both TAC1 hypermethylation frequency and mean NMV were higher in Bt than in Ba (75% versus 55.6% and 0.2313 versus 0.2145, respectively). Among 15 cases with corresponding normal esophagus, BE, and EAC, one (no. 2) was unmethylated, three (nos. 1, 3 and 13) were methylated only in EAC, three (nos. 5, 16, and 17) were methylated only in BE, and the remaining eight were methylated in both BE and EAC (Fig. 2A). Among 41 cases with corresponding normal esophagus and esophageal tumors, four (100%) of four cases (nos. 22, 23, 33, and 36) showing methylation in normal esophagus were also methylated in corresponding tumor (Fig. 2B), and the TAC1 NMVs of esophageal tumors (mean 0.209) were significantly higher than those of corresponding normal esophagus (mean 0.056; $P < 0.000001$, Student’s paired $t$ test; Fig. 2C). Twelve (50%) of 24 ESCC exhibited TAC1 hypermethylation.

BE was defined as long segment (LSBE) if it was $\geq 3$ cm in length, or short segment (SSBE) if $< 3$ cm, according to generally accepted criteria (16). The mean NMV of TAC1 was significantly higher in LSBE (mean 0.339) than in SSBE (mean 0.119; $P = 0.00544$, Student’s $t$ test, Fig. 3A). Similarly, the segment lengths of BEs with hypermethylated TAC1 promoters were significantly longer than the segment lengths of BEs with unmethylated promoters (mean 5.56 cm versus 2.25 cm; $P = 0.00256$, Student’s $t$ test; Fig. 3B), and the frequency of TAC1 hypermethylation was significantly higher in LSBE than in SSBE (87.5% versus 28.6%; $P = 0.0022$, Fisher’s exact test; Table 1).
Overall patient survival correlated with TAC1 methylation status in ESCC patients, but not in EAC patients (Fig. 3C and D). ESCC patients with hypermethylation of TAC1 (defined by a TAC1 NMV higher than the cutoff value of 0.12) had significantly shorter survivals than did patients without TAC1 hypermethylation (mean survival, 22 months versus 110 months; \( P = 0.0102 \), log-rank test; Fig. 3C).

No significant associations were observed between TAC1 promoter hypermethylation in tissues and patient age (data not shown), smoking or alcohol consumption status, tumor stage or lymph node metastasis, tumor differentiation, or histologic type of esophageal carcinoma (EAC versus ESCC; Table 1).

**TAC1 hypermethylation in freely circulating plasma DNA from patients of contrasting clinical statuses.** To further elucidate the potential value of TAC1 hypermethylation as a biomarker, we measured TAC1 methylation levels in 126 plasma samples from 35 control subjects, 10 additional patients with Ba, 20 with dysplastic Barrett’s esophagus, and 61 with EAC. The cutoff NMV (0.12) for dichotomization of plasma TAC1 data was chosen from the ROC curve using the following criteria: \( V \leq 10\% \) of plasma samples from control subjects hypermethylated and \( > 20\% \) of plasma samples from EAC patients hypermethylated. We chose achieving 90\% specificity as our top priority, based on the following reasoning: Additional true positives diagnosed by plasma methylated TAC1 levels represent new, potentially life-saving, usually asymptomatic diagnoses that would not have been made without the plasma assay. Thus, any true positive cases diagnosed in this manner constitute an improvement over current management. In contrast, false-positive diagnoses from plasma methylated TAC1 levels represent a potential detriment because they would result in unnecessary endoscopic procedures, needlessly increase costs, and lead to complications that would not have occurred without the plasma assay. Therefore, we considered it our highest priority to minimize false positives (i.e., to maximize specificity), even at the cost of diminishing true positives (i.e., sensitivity). Mean TAC1 hypermethylation NMVs and frequencies using the cutoff NMV of 0.12 in plasma samples are shown in Table 2. The TAC1 NMV in plasma was significantly higher in EAC patients (mean 0.2237) than in control subjects (mean 0.0336; \( P < 0.05 \), Student’s \( t \) test), patients with Ba (mean 0.0046; \( P < 0.05 \), Mann-Whitney \( U \) test), or patients with dysplastic Barrett’s esophagus (mean 0.0018; \( P < 0.01 \), Mann-Whitney \( U \) test). Similarly, TAC1 hypermethylation frequencies in plasma samples were significantly higher in EAC patients (29.5\%) than in control subjects (8.6\%; \( P < 0.05 \), Fisher’s exact test) or patients with dysplastic Barrett’s esophagus (0\%; \( P < 0.01 \), Fisher’s exact test), but not in patients with Ba (0\%; \( P = 0.056 \), Fisher’s exact test), yielding a sensitivity of 29.5\% (18 of 61) and a specificity of 91.4\% (32 of 35) for the diagnosis of EAC.

The methylation status of TAC1 in matched tissue and plasma samples are summarized in Supplementary Table S1. Among 17 control subjects with matched tissue and plasma samples, 15 cases with matched normal esophagus, BE, and EAC, one case (2) was unmethylated in all tissues, three (1, 3 and 13) were methylated (M) only in EAC, three (5, 16, and 17) were methylated only in BE, and the remaining eight were methylated in both BE and EAC. A, among 41 cases with matched normal esophagus and EAC, four of four cases (22, 23, 33, and 36) showing methylation in normal esophagus were also methylated in tumor. C. TAC1 NMVs of EAC (mean 0.209) were significantly higher than those of matched normal esophagi (mean 0.056; \( P < 0.0000001 \), Student’s paired \( t \) test).

**Fig. 2.** Methylation status of TAC1 in matched esophageal tissue samples. A, among 15 cases with matched normal esophagus, BE, and EAC, one case (2) was unmethylated in all tissues, three (1, 3 and 13) were methylated (M) only in EAC, three (5, 16, and 17) were methylated only in BE, and the remaining eight were methylated in both BE and EAC. B, among 41 cases with matched normal esophagus and EAC, four of four cases (22, 23, 33, and 36) showing methylation in normal esophagus were also methylated in tumor. C. TAC1 NMVs of EAC (mean 0.209) were significantly higher than those of matched normal esophagi (mean 0.056; \( P < 0.0000001 \), Student’s paired \( t \) test).
samples, 14 were unmethylated in both tissue and plasma, whereas three were methylated in plasma but unmethylated in tissue. Among 32 EAC patients with matched tissue and plasma samples, 10 were methylated in both tissue and plasma, 4 were unmethylated in both tissue and plasma, 14 were methylated in tissue but unmethylated in plasma, and 4 were unmethylated in tissue but methylated in plasma.

No significant associations were observed between TAC1 promoter hypermethylation in plasma samples and patient age (data not shown), survival (data not shown), tumor stage, or lymph node metastasis (Table 2).

**TAC1 methylation and mRNA levels in esophageal cancer cell lines after 5-Aza-dC treatment.** All 12 (three EAC and nine ESCC) esophageal cancer cell lines showed high TAC1 NMV levels, above the cutoff level of 0.12 (Fig. 4A). KYSE 220 and BIC, which exhibited some of the highest NMVs among the ESCC and EAC cell lines, respectively, were subjected to 5-Aza-dC treatment. After 5-Aza-dC treatment, the NMV of TAC1 was diminished, whereas the mRNA level of TAC1 was increased, in both KYSE220 and BIC cells (Fig. 4B).

**Discussion**

By alternatively spliced transcription, TAC1 encodes the neuropeptides substance P, neurokinin A, and neuropeptide K and γ, which act through two types of transmembrane G-protein–coupled receptors, denoted neurokinin-1 and neurokinin-2 (1). The precise involvement of TAC1 in carcinogenesis remains to be fully elucidated. The biological activity of TAC1 gene products may vary among different histologic tumor types (17). Substance P has been shown to have proliferative and antiapoptotic effects via the mitogen-activated protein kinase cascade and nuclear factor-κB (18, 19). Conversely, substance P inhibits melanoma formation in a murine model by a mechanism involving antitumor immunity (20). In the gastrointestinal tract, tachykinins regulate smooth muscle contractility, epithelial ion transport, vascular permeability, and immune function (21). The TAC1 gene locus shows a high frequency of loss of heterozygosity in EAC (2, 3). In the current study, we systematically investigated hypermethylation of the TAC1 gene promoter in primary human esophageal lesions of differing histologic types and grades. Our results show that TAC1 promoter hypermethylation occurs frequently in both human EAC and ESCC. The frequency of TAC1 hypermethylation was extremely low in normal esophagus but increased at the very early preneoplastic stage of Ba, while being maintained in dysplastic Barrett’s esophagus and EAC. There was no significant association between TAC1 promoter hypermethylation and histologic subtype of esophageal carcinoma (EAC versus ESCC). These results suggest that hypermethylation of TAC1 occurs early in many subjects, that the frequency of this epigenetic event increases during esophageal carcinogenesis,
particular in EAC, and that this event is highly prevalent in human esophageal cancers.

Conflicting results regarding the length of BE as a predictive factor in neoplastic progression have been reported. Although some previous studies stated that patients with SSBE can develop dysplasia (22) and EAC (16), several prospective studies showed an increased EAC risk only with LSBE (23–25). In a prospective cohort study of 309 BE patients followed in the Seattle Barrett’s Esophagus Project, segment length was not related to cancer risk (P > 0.2); however, when patients with high-grade dysplasia at entrance were excluded, a trend was indeed observed, with a 5 cm difference in length associated with a 1.7-fold increase in cancer risk (95% confidence interval, 0.8-fold to 3.8-fold; ref. 16). Weston et al. (23) reported significant differences in the frequencies of both dysplasia and EAC between SSBE and LSBE, at 8.1% versus 24.4% for dysplasia (P < 0.0001) and 0% versus 15.4% for EAC (P < 0.0005). Hirota et al. (24) reported that the prevalence of dysplasia and cancer differed significantly between patients with SSBE and patients with LSBE in a comprehensive prospective study of 889 consecutive subjects. More recently, Hage et al. (25) reported a significantly increased risk of progression to high-grade dysplasia or EAC with LSBE after a mean follow-up period of 12.7 years. Thus, it seems likely that length of Barrett’s epithelium is a contributory risk factor for both the prevalence (presence) and incidence (future development) of dysplasia and EAC. In the current study, TAC1 methylation manifested a strong relationship to BE segment length. There were significant associations between TAC1 promoter hypermethylation as a binary variable and BE segment length as a continuous variable (P = 0.00256), as well as between TAC1 promoter hypermethylation as a continuous variable and BE segment length as a binary variable (P = 0.00544). The frequency of TAC1 hypermethylation was significantly higher in LSBE (87.5%) than in SSBE (28.6%; P = 0.0022). Thus, TAC1 methylation may constitute a molecular correlate of BE segment length, in addition to its potential value as a biomarker for the prediction of BE progression.

There is increasing evidence that promoter hypermethylation has prognostic value in cancer patients, including those with esophageal cancer (12, 26, 27). Brock et al. (26) showed that hypermethylation of multiple genes was a powerful indicator of poor prognosis in EAC patients. Lee et al. (27) reported that the Fragile Histidine Triad gene was hypermethylated in 85 (33%) of 257 ESCC and associated with a poor prognosis in stage I to II cases. Furthermore, our previous work showed that hypermethylation of adenomatous polyposis coli gene DNA could be detected in circulating plasma and was associated with a poor prognosis in EAC patients (12). In the current study, TAC1 hypermethylation in tissue was significantly associated with shortened survival in ESCC patients, and both mean NMV and frequency of TAC1 hypermethylation in plasma were significantly higher in EAC patients than in control subjects. Thus, hypermethylation of TAC1 seems to constitute a potentially useful biomarker of biologically aggressive disease in ESCC and EAC patients. Although TAC1 hypermethylation in plasma DNA was observed predominantly in EAC patients, 8.6% (3 of 35) of control subjects in the current study manifested plasma TAC1 hypermethylation. In addition, four EAC patients with plasma TAC1 hypermethylation did not show the same alteration in their matched tumor tissues. We considered the following possible explanations for these results: (a) methylated plasma DNA could have been derived from undetected precancerous lesions in these cases; (b) apparent plasma false-positive control subjects could actually possess an increased predilection to develop malignant disease in the future; or (c) malignancies already present in other unscreened organs could have gone undetected at the time point analyzed.

In the current study, reversal of methylation and restoration of TAC1 expression occurred in both KYSE220 and BIC esophageal cancer cell lines after 5-Aza-dC treatment.
Restoration of TAC1 mRNA expression by 5-Aza-dC treatment is consistent with the interpretation that DNA hypermethylation was responsible for silencing of TAC1.

The current study suggests that hypermethylation of the TAC1 promoter, leading to gene silencing, is a common event in human esophageal carcinomas, occurs early in Barrett’s-associated esophageal adenocarcinogenesis, and is associated with a poor prognosis in ESCC patients.

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References

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