Advances in Brief

AIS Overexpression in Advanced Esophageal Cancer

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Abstract

We examined AIS status in digestive tract cancers and found that all eight esophageal cancer cell lines (100%) showed AIS/TA-AIS gene overexpression, whereas 1 of 12 (8%) gastric cancer and 0 of 14 (0%) colon cancer cell lines showed AIS/TA-AIS gene expression. We then confirmed that the AIS gene, not the TA-AIS gene, was dominantly expressed in esophageal cancers by reverse transcription-PCR. AIS protein was also expressed in AIS gene-positive cell lines. Subsequently, we tested AIS gene expression in paired esophageal normal tissues and cancers. Twenty-five of 39 (64%) primary esophageal cancers demonstrated an obviously higher expression of AIS gene compared to paired normal tissues. Moreover, high AIS gene expression was significantly associated with lymph node metastases in esophageal cancer (P = 0.0271). These results suggested that AIS may be useful as a marker for advanced esophageal cancer.

Introduction

p53 is the most commonly inactivated gene in human cancer, and the loss of critical p53 pathways is central to tumorigenesis (1–3). Recently, a new human p53 homologue, p40, was isolated using a degenerate PCR approach that showed that p40 was located on chromosome 3q28 (4). The DNA binding domain and the oligomerization domain of p40 display a strong conservation of amino acid residues with p53, raising the possibility that human p40 may also bind key p53 DNA binding sites in the human genome and/or interact with p53. Concurrently, an alternative p40 transcript termed p51 was cloned and shown to suppress colony formation in cell lines and to transcriptionally activate p21 in a fashion similar to the p53 tumor suppressor gene (5). Another group of splice variants (p63) was also described (6). A transcript that lacked the NH2-terminal TA domain of p53 (∆Np63) was found to act in a dominant negative fashion and to be able to suppress p53 transactivation. The recent cloning of these variants has led to a potentially confusing nomenclature. We could functionally divide these variants into two groups by the presence of the TA domain (Fig. 1).

We further examined p40 status (now called AIS because this gene was frequently amplified in squamous cell carcinoma; Fig. 1), and observed AIS overexpression in head and neck cancer cell lines and primary lung cancers associated with a low-level increase in chromosomal copy number (7). Moreover, we found that increased expression of AIS in mouse fibroblast cells led to a transformed phenotype. Conversely, no evidence of a tumor-suppressive function of AIS in these cancers was found. Our data suggested that AIS may play an oncogenic role in certain cancers.

These results prompted us to examine AIS status in digestive tract cancers, especially esophageal cancer, which is one of the most common squamous cell cancers. In this study, we examined AIS expression in digestive tract cancer cell lines using Northern analysis. Interestingly, all esophageal cancer cell lines showed AIS overexpression. Besides, AIS expression was significantly related to lymph node metastases of primary esophageal cancer, suggesting that AIS may be a new marker for advanced esophageal cancer.

Materials and Methods

Northern Analysis. Seven cell lines were established in our laboratory (NUEC1, NUEC2, NUEC3, NUEC4, NUGC2, NUGC3, and NUGC4). The other cell lines were kindly provided by the Memorial Sloan-Kettering Cancer Center (New York, NY) or purchased from the American Type Culture Collection (Manassas, VA). Cultured cell lines were lysed in guanidine buffer, and total RNA was isolated using the CsCl gradient method. For primary tissues, the collected samples were grossly dissected and quickly frozen or lysed immediately in the guanidine buffer, and the RNA was isolated using the CsCl gradient method.

First-strand cDNA was generated from RNA by reverse transcription (RT-PCR).^2^ The primary PCR amplification consisted of 30 cycles (95°C for 30 s, 55°C for 1 min, and 72°C for 1 min) after the initial denaturation step (95°C for 2 min). The primers

^2^ The abbreviation used is: RT-PCR, reverse transcription-PCR.
used were as follows: (a) AIS-S (sense), 5'-GCAGCATTGATCAATCTTACAG (b) TA-AIS-S (sense), 5'-CAAGATTGAGATTAGCATGGAC; and (c) AIS-AS (antisense), 5'-TGAATTTCACGGCTCAGCTCAT. These primers are shown in Fig. 1.

The PCR amplification for AIS gene (AIS-S and AIS-AS) or TA-AIS gene (TA-AIS-S and AIS-AS) was performed as described previously (11). The predicted size of PCR products from the AIS and TA-AIS genes was 505 and 500 bp, respectively.

Western Analysis. Cells from a T-75 flask were trypsinized and washed with PBS, suspended in 200 μl of RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride, and then incubated on ice for 20 min. The lysate was centrifuged at 10,000 rpm for 10 min, and the supernatant was stored at −80°C. Fifty μg of cell lysates were separated on a 12% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, United Kingdom). After blocking the non-specific sites by incubation in PBS 1% nonfat dry milk, the blot was incubated with monoclonal anti-p63 antibody (4A4; Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a 1:200 dilution for 1 h at room temperature. This antibody is broadly reactive with all known p63 variants. After washing, an enhanced chemiluminescence kit (Amersham Pharmacia Biotech UK Ltd.) was used to visualize the antibody binding to each protein. The molecular size of AIS protein was 68 kDa, as described previously (7).

Statistical Analysis. The χ² test was used to examine the association between AIS expression and clinicopathological features.

Results

We first examined AIS/TA-AIS gene expression in esophageal, gastric, and colon cancer cell lines by Northern analysis using a common cDNA probe to AIS and TA-AIS genes. We found that all 8 (100%) esophageal cancer cell lines showed AIS/TA-AIS gene overexpression, whereas 1 of 12 (8%) gastric cancer and 0 of 14 (0%) colon cancer cell lines showed AIS/TA-AIS gene expression (Fig. 2, A–C). Subsequently, we examined the type of AIS gene (AIS or TA-AIS) by RT-PCR that has been amplified in these cancer cell lines. According to the result from our previous AIS work, AIS is dominantly expressed in some primary lung cancers and head and neck cancer cell lines (7). As expected, all cell lines that demonstrated AIS/TA-AIS gene overexpression in Northern analysis showed AIS gene expression, whereas only one cell line (NUEC3) expressed the TA-AIS gene. A human β-actin probe was used as an internal control. AIS protein expression was also observed in all cell lines by Western analysis. Moreover, RT-PCR showed that AIS/TA-AIS transcripts existed in all esophageal cancer cell lines, whereas only one cell line (NUGC2) expressed the TA-AIS gene. A human β-actin probe was used as an internal control. AIS protein expression was also observed in all cell lines by Western analysis. Moreover, RT-PCR showed that AIS/TA-AIS transcripts existed in all esophageal cancer cell lines.

To confirm the results, we further examined AIS protein expression in these cell lines using Western analysis. All esophageal cancer cell lines and one gastric cancer cell line that had

**Fig. 1** AIS gene variants. Although AIS and TA-AIS genes have the same core domain, AIS lacks the transactivating domain (TA domain), whereas the TA-AIS gene conserves it. The cDNA probe to AIS and TA-AIS genes is represented by a black bar. PCR primers used for amplification of the AIS and TA-AIS genes are indicated by arrows.

**Fig. 2** Detection of AIS expression in digestive tract cancers. A, AIS expression in esophageal cancer cell lines. AIS/TA-AIS and protein expression was observed in all esophageal cancer cell lines, whereas only one cell line (NUEC3) expressed the AIS gene. Moreover, RT-PCR showed that AIS/TA-AIS transcripts existed in all esophageal cancer cell lines. A human β-actin probe was used as an internal control. AIS protein expression was also observed in all cell lines by Western analysis. B, AIS expression in gastric cancer cell lines. AIS/TA-AIS and protein expression was observed only in NUGC2, as confirmed by RT-PCR. Although RT-PCR showed that TA-AIS transcripts existed in MKN1, no expression was observed by Northern or Western analysis. C, no AIS expression was seen in colon cancer cell lines.
AIS gene expression also showed AIS protein expression (Fig. 2, A–C).

We then tested AIS/TA-AIS gene expression in paired esophageal normal tissues and cancers. Normal esophageal tissues showed low-grade expression of AIS/TA-AIS gene compared to paired normal tissues (Fig. 3). We also examined the type of AIS gene by RT-PCR and found that AIS gene was expressed in both normal esophageal tissues and cancers, whereas no TA-AIS gene was expressed (Fig. 3). To examine a possible relationship between AIS gene overexpression and clinicopathological features, we proceeded with statistical analysis. Interestingly, high AIS gene expression was significantly associated with lymph node metastases in esophageal cancer (P = 0.0271; Table 1). AIS gene overexpression also showed a tendency to correlate with tumor-node-metastases (TNM) stage (P = 0.0727). These results suggest that AIS may be useful as a marker for advanced esophageal cancer.

Discussion

Esophageal cancer is one of the most aggressive cancers, and it occurs at a high incidence in certain countries (12). To eliminate this fatal cancer from patients, we perform surgical operations and subsequent chemotherapy and radiotherapy. For this purpose, it is critical to estimate the malignancy of cancer.

Accumulating evidence indicates that a series of genetic changes in dominant oncogenes such as bcl-2, cyclin D1, and c-myc together with the inactivation of tumor suppressor genes such as p53 are involved in the pathogenesis of human esophageal cancer (1, 13–15). Several other candidate oncogenes have also been implicated in reports (8, 16) indicating that bcl-2 protein expression was more frequent among poorly differentiated tumors than among well-differentiated tumors. However, no correlations were found between bcl-2 protein expression and the following parameters: (a) tumor size; (b) depth of invasion; and (c) nodal status. On the other hand, int2/hst1 coamplification was significantly correlated with a high incidence of eventual metastasis in distant organs. In the present study, we first detected overexpression of the AIS gene using esophageal cancer cell lines. We further examined the relationship between AIS overexpression and clinicopathological features using 39 primary esophageal cancers and found that AIS gene expression was significantly related with lymph node metastases in this cancer.

Although other splice variants with a TA domain have been shown to be growth suppressive, our previous results do not support a tumor suppressor role for AIS in head and neck and lung cancers (7). In this study, we further examined AIS gene expression using digestive tract cancers and demonstrated that all esophageal cancer cell lines and advanced esophageal cancers show overexpression of the AIS gene. Our data for AIS gene overexpression are consistent with two recent reports indicating the presence of an amplification of chromosome 3q in esophageal cancer. Both studies used comparative genomic hybridization to examine chromosomal gains in primary esophageal cancers and showed that 50% and 72% of esophageal cancers have a chromosome 3q gain (17, 18).

Although the precise mechanism of AIS on oncogenicity remains to be proven, it was confirmed that AIS gene expression may be a new marker for advanced esophageal cancer. Work in progress suggests a direct protein-protein interaction between AIS and p53 and may well someday soon explain the diminution of p53 transactivation in the future.

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References


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