Loss of H19 Imprinting in Esophageal Cancer

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Abstract

Recent articles have reported that loss of imprinting (LOI) of the endogenous gene H19 was frequently found in lung cancer and choriocarcinoma, common adulthood cancers. Consequently, we examined the status of genomic imprinting of H19 in 29 esophageal and 48 colorectal cancer specimens, and studied its relation to the expression of H19. Of 12 esophageal cancer specimens heterozygous for the RsaI polymorphism, 6 (50%) exhibited LOI of H19, but none of the 18 colorectal cancer specimens heterozygous for the RsaI polymorphism exhibited LOI of H19. The present study suggests that LOI of H19 may play an important role in the pathogenesis of esophageal cancer. Moreover, H19 expression was frequently abundant in both cancers, and all six esophageal cancers carried LOI with overexpressed H19. Therefore, this overexpression of H19 seems to be an important phenomenon for the development of esophageal and colorectal cancer cells.

Introduction

Genomic imprinting is a parental origin-specific chromosomal modification that causes differential expression of maternal and paternal genes. H19 is an endogenous gene showing maternal-specific monoallelic expression on 11p15 (1). H19 is one of the most abundant mRNAs in the fetus and the placenta, and presumably acts as an RNA molecule; the H19 gene does not code for a protein (2–4). Recent articles have reported that LOI of H19 was identified to be frequent not only in childhood tumors such as Wilms’ tumor and hepatoblastoma (5, 6) but also in lung cancer and choriocarcinoma, common adulthood cancers (7, 8). LOI may be an example of gene activation by overexpression of the growth promoter, in that a disturbance of genomic imprinting in the tumor causes activation of the transcriptionally repressed copy (9). LOI thus plays a part in genetic abnormalities such as point mutation, rearrangement, deletion, and amplification.

Recently, two independent groups have reported significantly reduced expressions of H19 in Wilms’ tumor, lending support to the hypothesis that H19 may act as a tumor suppressor gene for such lesions (10, 11). On the contrary, LOI in hepatoblastoma was not associated with the down-regulation of H19 (6), and LOI of H19 was often associated with its overexpression in human lung cancer (7). It would appear that H19 might have differential roles depending on the tissue type as well as its stage of development.

Mounting evidence indicates that digestive tract cancers, which are common adulthood cancers, carry many genetic alterations. However, LOI and expression of H19 have not been reported in them until now. In the present study, we examined 29 esophageal and 48 colorectal cancer specimens to investigate the status of H19 genomic imprinting as well as its relation to the expression of H19. Marked LOI was observed for H19 in esophageal cancers in contrast to no LOI in colorectal cancers. The H19 gene was frequently overexpressed in both cancers compared to corresponding normal tissues, and LOI of H19 was always associated with its overexpression.

Materials and Methods

Tumor Samples. Tumor samples along with corresponding normal tissues were collected at the Nagoya University School of Medicine and Okazaki Municipal Hospital from 29 esophageal and 48 colorectal cancer patients who were diagnosed histologically. These samples were obtained during surgery. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until they were analyzed. DNA was prepared by extraction with phenol, while RNA was extracted with guanidium thiocyanate followed by ultracentrifugation in cesium chloride solution.

Analysis of Allele-specific Expression of H19 Using an RsaI Polymorphism. An RsaI polymorphism in the 3’ last exon of H19 was used to analyze allele-specific expression (1). We first screened for esophageal and colorectal cancer specimens heterozygous for the RsaI polymorphism using PCR amplification of genomic DNA followed by complete digestion with 10 units RsaI and electrophoresis on a 2% agarose ethidium bromide gel. The PCR amplification consisted of 35 cycles (94°C for 1 min, 65°C for 3 min, and 72°C for 4 min) after the initial denaturation step (94°C for 5 min). The primers used were: S1 (sense), 5’-TACAACCACTGCACTACCTG and AS2 (antisense), 5’-TGAATTCTTGAGGCTGT. Informative samples were further analyzed for allele-specific expression using PCR products of the first-strand cDNA, as described above. First-strand cDNA synthesis and subsequent PCR amplification were performed as described previously (12). In heterozygotes, RsaI digestion of PCR products of first-strand cDNA yielded 575-bp and 407 + 168-bp fragments, which were slightly smaller than those observed in the analysis of genomic DNAs because of the presence of an 80-bp intron. All patterns were reproducible in each repeated assay.

Northern Blot Analysis. Ten µg total RNA were electrophoresed on a 1% agarose gel containing formaldehyde and transferred to a Gene Screen (DuPont), as described previously (13). It was then consecutively hybridized with the H19 and β-actin probes. The H19 probe was generated by reverse transcription-PCR using primers S1 and AS2, as described above. A human β-actin probe was used as an internal control (14). Using appropriate exposure of the autoradiograms, the signal intensity was determined by computer software, NIH image.

Results and Discussion

We examined the status of genomic imprinting of the H19 gene in 29 esophageal and 48 colorectal surgical specimens by reverse transcription-PCR analysis using an RsaI polymorphism in the last exon. Of 12 cases identified as heterozygotes for the RsaI polymorphism, 6 (50%) exhibited LOI of H19 (Fig. 1). In marked contrast, 0 of the 18 colorectal cancer specimens heterozygous for the RsaI polymorphism exhibited LOI of H19 (Fig. 1). We also examined whether there are changes in imprinting of IGF2, which is another imprinting gene and often coordinately regulated with H19, in these esophageal cancer specimens. No specimens exhibited LOI of IGF2 according to cancerous change. So we think that alteration of H19 is an independent phenomenon from the status of IGF2 in esophageal cancers (data not shown).

We performed Northern blot analysis to investigate the difference of the H19 expression level between normal and tumor samples. Of the 16 esophageal and 48 colorectal cancer samples, the expression of
**LOSS OF H19 IMPRINTING IN ESOPHAGEAL CANCER**

**Esophageal Cancer**

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**Colorectal Cancer**

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Fig. 1. A representative autoradiograph showing monoallelic and biallelic expression of H19 in esophageal cancer specimens and monoallelic expression in colorectal cancer specimens heterozygous for the Rsa I polymorphism. In contrast to monoallelic expression in the corresponding normal (N) esophageal samples, LOI is observed in tumor (T) RNAs of cases E22, E26, and E27. In contrast to biallelic expression of esophageal samples, colorectal samples show only monoallelic expression.

*H19* higher than that in corresponding normal tissues was observed in 12 (75%) esophageal and 31 (65%) colorectal cancer samples (expression relative to corresponding normal tissues, > 1.5), while no cancer samples showed expressions lower than that in paired normal tissues (Fig. 2). We observed that *H19* expression was frequently abundant in both cancers, and all six esophageal cancers carried LOI with overexpressed *H19* (expression relative to corresponding normal tissues, > 1.9). LOI and the relative *H19* expression level in esophageal cancers are summarized in Table 1.

We found that LOI of *H19* was associated with one half of our esophageal cancer specimens. In esophageal cancer, LOI of *H19* seems to be more frequent than Wilms' tumor (29%) (5) and lung cancer (38%) (7), but as frequent as primary choriocarcinoma (50%) (8). The present study suggests that LOI of *H19* may play an important role in the pathogenesis of esophageal cancer. On the other hand, no LOI of *H19* has been found in colorectal cancer, which is the same digestive tract cancer as esophageal cancer; consequently, we think LOI of *H19* may be a specific link with esophageal cancer. In contrast to this high frequency of LOI, none of the esophageal cancer specimens exhibited LOH. Moreover, frequent occurrence of LOI of *H19* was associated with the overexpression in esophageal cancer which was similar to lung cancer (7). These results are in direct contradiction to a tumor suppressor gene role involved in the pathogenesis of this common cancer in adults (15). Therefore, as Kondo et al. (7) suggested, *H19* might have differential roles, depending on the tissue type as well as the developmental stage, and changes in the stringent regulation of *H19* due to LOI may confer an unexpected growth advantage rather than growth retardation on esophageal cancer cells. In this regard, it is interesting to note that the esophagus and lung are derived from the same embryological origin, the foregut, and colon is from the midgut and hindgut. Indeed, there are also some common genetic alterations between esophageal and lung cancers such as LOH on chromosome 3p (16, 17).

Both esophageal and colorectal cancers frequently exhibit overexpressions of *H19*, in spite of whether LOI exists or not. Therefore, there is not a statistically significant difference for the *H19* expression level between the cases showing LOI of *H19* and the cases that do not (P = 0.18, Fisher's exact test), although all six esophageal cancers with LOI overexpressed *H19*. So we think, since the rapidly growing fetus needs abundant *H19* mRNAs, this overexpression of *H19* is an important phenomenon for the development of esophageal and colorectal cancer cells, and LOI may be one important process which produces an overexpression of *H19*. In the future, if *H19* expression is examined by *in situ* hybridization, *H19* expression may be used as a tumor marker in esophageal and colorectal cancers similar to that in bladder cancer (18).

Although the precise mechanism of LOI of *H19* on gene expression remains to be proven, it was confirmed that LOI of *H19* is an
organ-specific genetic change, and that H19 overexpression may play an important role in the progression of esophageal and colorectal cancers. Additional studies, such as those used for assessing the expression level of H19 in precancerous regions, are required to gain an insight into neoplastic processes in the esophagus and colon.

Acknowledgments

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