DNA Hypermethylation at the D17S5 Locus in Non-Small Cell Lung Cancers: Its Association with Smoking History

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ABSTRACT

The aim of this study was to examine the association between DNA hypermethylation and clinicopathological features of non-small cell lung cancers (NSCLCs). The DNA methylation status at the D17S5 loci, at which a candidate tumor suppressor gene, HIC-1 (hypermethylated in cancer), was identified, of 51 paired tumor and nontumorous lung tissue specimens from NSCLC patients was examined by Southern blot analysis, using a methylation-sensitive restriction enzyme. DNA hypermethylation at this locus was found in 17 (33%) tumors and 16 (31%) nontumorous lung tissues. DNA in hypermethylation at this locus occurred more frequently in poorly differentiated tumors, especially in adenocarcinomas, and correlated significantly with the loss of heterozygosity at this locus in tumors (P = 0.01). The incidence of DNA hypermethylation was significantly higher in smokers than those who had never smoked in both tumors and nontumorous lung tissues (P = 0.03 and P = 0.01, respectively). These results suggest that DNA hypermethylation at the D17S5 locus may play a role in the development of NSCLCs in cigarette smokers.

INTRODUCTION

Alterations in DNA methylation at cytosine residues of CpG sites may play roles in carcinogenesis from three aspects (1–4): (a) DNA methylation facilitates point mutations of genes, because 5-methylcytosine is deaminated to thymine (5, 6); (b) DNA methylation in clusters of CpG dinucleotides near the regulatory regions of genes affects the transcription of specific genes (7–14); and (c) regional DNA methylation alterations cause chromatin configuration changes (15–18).

DNA hypermethylation changes of the Nol sites at the D17S5 locus in colon cancers (19), neural tumors (17), renal cancers (18), and ovarian cancers (20) have been reported, and Makos et al. (17, 18) suggested that DNA hypermethylation at the D17S5 loci in these tumors predisposes this locus to LOH. Furthermore, a candidate tumor suppressor gene, HIC-1, was identified in a region encompassing the aberrantly methylated Nol sites in these tumors (21). The HIC-1 gene was methylated and was either not expressed or was expressed at a very low level in astrocytomas, colon cancers, breast cancers, and small cell lung cancer cell lines, whereas it was not methylated and ubiquitously expressed in normal brain, colon, and lung tissues, suggesting that the HIC-1 gene is silenced by DNA hypermethylation (21).

To the best of our knowledge, no detailed investigations into correlations between DNA hypermethylation at this locus and clinicopathological features of lung cancers, especially NSCLCs, have been reported. In an attempt to examine the association between DNA hypermethylation and clinicopathological features of NSCLCs, we assessed the DNA methylation status at the D17S5 loci of 51 paired tumor and nontumorous lung tissue specimens from NSCLC patients.

MATERIALS AND METHODS

Tissue Samples and DNA Extraction. Peripheral lung tumors and nontumorous lung tissues, which were at least 4 cm distant from tumors macroscopically, were obtained from surgically resected specimens of 51 patients (cases 1 to 51; 32 males and 19 females; mean age, 61.4 ± 8.2 years) who underwent operations in the National Cancer Center Hospital, Tokyo, Japan, in 1996. Thirty-five of the patients had continued to smoke up to the period of preparation for surgery, and the other 16 had never smoked. Patients who stopped smoking before the preoperative preparation period were not found in this study, and those with a history of specific occupational exposure, chronic lung disease, and/or had received preoperative treatment for cancer were excluded. The NSCLCs were classified histologically according to the WHO criteria (22). High molecular weight DNA was isolated from fresh tissue samples by phenol-chloroform extraction and membrane dialysis (23).

Methylation Assay. Five-μg aliquots of genomic DNA were digested for 24 h by the methylation-sensitive restriction enzyme Nol (Toyobo, Tokyo, Japan) at 37°C, electrophoresed, transferred to nitrocellulose membranes, and hybridized with probe YNZ22.1 (provided by the American Type Culture Collection), which was reported to reveal highly polymorphic loci (24). According to the criteria described previously (19), when polymorphic DNA bands of approximately 4.5–5.0 kb were identified, the CpG sites around the D17S5 locus were judged unmethylated, and when 6.0–8.0-kb or 20-kb bands were identified, the CpG sites were judged methylated.

LOH Analysis. Five-μg aliquots of genomic DNA were digested for 24 h by the restriction enzyme TaqI (Toyobo) at 65°C, electrophoresed, transferred to nitrocellulose membranes, and hybridized with probe YNZ22.1 (24). According to the criteria described previously (19), when polymorphic DNA bands of approximately 4.5–5.0 kb were identified, the CpG sites around the D17S5 locus were judged unmethylated, and when 6.0–8.0-kb or 20-kb bands were identified, the CpG sites were judged methylated.

RESULTS

DNA Hypermethylation and LOH in Tumors. DNA hypermethylation at the D17S5 locus was observed in 17 (33%) of the 51 lung tumors examined (Fig. 1A and Table 1). The incidences of DNA hypermethylation were higher in tumors with large (>30 mm) than small (<30 mm) maximum diameters, and in tumors with than without lymph node metastasis, although these differences failed to reach significance. The incidence of DNA hypermethylation was higher in poorly than moderately or well-differentiated squamous cell carcinomas, although this difference also failed to reach significance. The incidence of DNA hypermethylation in adenocarcinomas correlated significantly with the differentiation grade (P = 0.01; Table 1).

LOH at the D17S5 locus was observed in 18 (56%) lung tumors obtained from 32 informative cases (Fig. 1B), and DNA hypermethylation and LOH at the D17S5 locus correlated significantly (P = 0.01; Table 2).

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3 The abbreviations used are: LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer.
DNA hypermethylation in smoking-associated lung cancers. DNA hypermethylation at the D17S5 locus was observed in 16 (31%) of the 51 non-tumorous lung tissue samples examined (Table 3). There was no correlation between patient age and DNA methylation status at this locus in non-tumorous lung tissues (data not shown). Non-tumorous lung tissues in which DNA hypermethylation was detected included the tissues obtained from 4, 10, and 2 patients with squamous cell carcinomas, adenocarcinomas, and others, respectively. In 8 of 16 (50%) patients with DNA hypermethylation in the corresponding non-tumorous lung tissues, DNA hypermethylation was also observed in their tumors, whereas only 9 (26%) of 35 without DNA hypermethylation in the former showed DNA hypermethylation in the latter.

Correlations between DNA hypermethylation and smoking habits. The incidence of DNA hypermethylation at the D17S5 locus in both the tumors and the non-tumorous lung tissues correlated significantly with the smoking habit (P = 0.03 and P = 0.01, respectively; Table 3).

DISCUSSION

In this study, we demonstrated a high frequency of DNA hypermethylation at the D17S5 locus in NSCLCs. The incidence of DNA hypermethylation was higher in tumors with large than in those with small maximal diameters, and in tumors with than in those without lymph node metastasis. The DNA hypermethylation incidence correlated significantly with the differentiation grade, which has been reported to be a prognostic factor for adenocarcinomas of the lung (27, 28). These data suggest that DNA hypermethylation at this locus is closely associated with a high grade of malignancy of NSCLCs.

Makos et al. (17, 18) indicated that DNA hypermethylation at the D17S5 locus predisposes to chromatin configuration changes, resulting in LOH on 17p in neural (17) and renal (18) tumors. In agreement with these reports, our study revealed that DNA hypermethylation at the D17S5 locus was often accompanied by allelic loss at this locus. Therefore, DNA hypermethylation at the D17S5 locus may participate in NSCLC progression by predisposing this locus to LOH and/or silencing the HIC-1 gene.

Although Makos et al. (19) reported that DNA hypermethylation at the D17S5 locus was not detected in normal lung tissue samples, Vertino et al. (29) indicated that human uncultured bronchial epithelial cells obtained from an autopsy case showed a partially methylated pattern at the D17S5 locus. Furthermore, Vertino et al. (29) proposed that DNA hypermethylation at this locus was an early event in bronchial cell carcinogenesis, because DNA hypermethylation patterns became more extensive after immortalization and oncogene-
induced neoplastic transformation of the bronchial epithelial cells, and speculated that the preexisting partial methylation in uncultured bronchial epithelial cells from an autopsy case may have been caused by exposure to an environmental carcinogen. In accordance with this report by Vertino et al. (29), DNA hypermethylation at the D17S5 locus was observed in 83% of nontumorous lung tissues, which may contain NSCLC progenitor cells, from our cohort of NSCLC patients, suggesting that DNA hypermethylation at this locus participates in the early developmental stages of NSCLCs.

Furthermore, the molecular weights of the NotI-digested DNA fragments from tumors were higher than those of nontumorous lung tissues, and the intensities of the larger-sized bands from tumors were greater than those of nontumorous lung tissues. These findings reflect apparent progressive increases in the numbers of methylated CpG dinucleotides and cells showing DNA hypermethylation during the progression from precancerous conditions to NSCLCs.

In conclusion, DNA hypermethylation at the D17S5 locus during the development of NSCLCs.

In the clinical specimen, DNA methylation may have the potential to be a new strategy for prevention of, or therapy for, NSCLCs, especially in cigarette smokers.

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