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No Evidence of Mutation in the Human *PTC* Gene, Responsible for Nevoid Basal Cell Carcinoma Syndrome, in Human Primary Squamous Cell Carcinomas of the Esophagus and Lung

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The high frequency of loss of heterozygosity that has been observed on the distal region of the long arm of chromosome 9 in squamous cell carcinomas of esophagus, lung, uterus, and head and neck indicates the presence of a tumor suppressor gene(s) in this region. To investigate the possible role of the *PTC* gene on chromosome 9q22.3, that was identified as the cause of nevoid basal cell carcinoma syndrome, during carcinogenesis in esophagus and lung, we examined 20 esophageal squamous cell carcinomas and 10 squamous cell carcinomas of the lung for mutations in any coding exon of *PTC*. Using single-strand conformation polymorphism and direct sequencing, we detected no mutations other than two non-deleterious polymorphisms. Our results suggest that inactivation of some tumor suppressor gene(s) on 9q other than *PTC* contributes to the development of squamous cell carcinomas in these tissues.

Key words: Squamous cell carcinoma — *PTC* gene — Chromosome 9q

Nevoid basal cell carcinoma syndrome (NBCCS, or Gorlin syndrome) is a cancer predisposition characterized by multiple basal cell carcinomas and diverse developmental abnormalities. After the gene responsible for NBCCS was localized on the long arm of chromosome 9 by linkage analysis in families carrying the syndrome,[1,2] the *PTC* gene on chromosome 9q22.3,[3] became a likely candidate. Subsequently, analysis of DNA from patients with NBCCS, as well as DNA from sporadic basal cell carcinomas, identified germline and somatic mutations of *PTC*.[3–5] Inactivation of this gene therefore appears to play a significant role in development of basal cell carcinomas.

Loss of heterozygosity (LOH) analyses of squamous cell carcinomas of the esophagus, lung, urinary bladder, and head and neck have revealed a high frequency of allelic losses (LOH) on the distal long arm of chromosome 9, a finding that implies the presence of a tumor suppressor gene(s) in this chromosomal region.[6–9] Although we have already narrowed down the commonly deleted region in squamous cell carcinoma of the esophagus to 9q31-32, some tumors revealed no LOH at 9q31-32, but showed LOH in a more proximal region.[6] Hence, we considered the possibility that inactivation of the human *PTC* gene might play an important role in development of some sporadic cases of squamous cell carcinomas of the esophagus and lung, and screened its entire coding region for mutations in 20 esophageal and 10 lung carcinomas.

Tumor samples were obtained from patients with esophageal or lung cancers during surgery at the Cancer Institute Hospital (Tokyo), the Tohoku University Hospital (Miyagi), the Fourth Affiliated Hospital of Hebei Medical College (Hebei, China), or the Saitama Cancer Center (Saitama). Upon removal, samples were frozen in liquid nitrogen. The 20 esophageal tumors were histopathologically diagnosed as squamous cell carcinomas. Of the 10 lung tumors, five were diagnosed as squamous cell carcinomas, and the others as adenosquamous cell carcinomas. DNAs were extracted from the tissues according to methods described previously.[10]

To screen tumor DNAs for variant sequences within the *PTC* gene, single-strand conformation polymorphism (SSCP) analysis was performed as follows. Each 10-μl reaction mixture contained 20 ng of genomic DNA, 25 m*M* dNTPs, 0.05 U of ExTaq DNA polymerase, 25 n*M* of each oligonucleotide in 10 m*M* Tris (pH 8.8), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, and 0.2 μl of [α-^{32}P]dCTP (3000 Ci/mmol, Amersham Japan, Tokyo). Amplification was done with 35 cycles of 94°C, 55°C, and 72°C for 0.5, 0.5, and 1 min, respectively, in a thermal cycler (Perkin Elmer-Cetus 9600, Foster City, CA).

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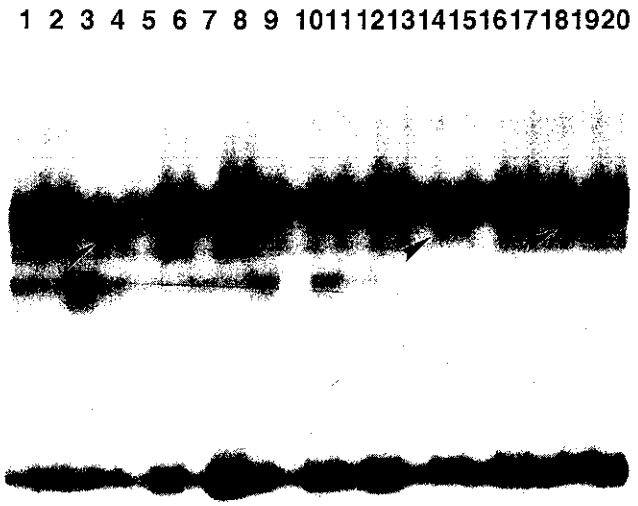

Fig. 1. Single-strand conformation polymorphism analysis of exon 17 of the human *PTC* gene in DNAs from 20 squamous cell carcinomas of the esophagus. The bands showing aberrant mobilities (cases 4, 15, and 19) are indicated by arrows.

After the PCR, 10 μl of each reaction mixture was transferred into 10 μl of loading buffer (95% formamide, 20 m*M* EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). The samples were heated at 80°C for 10 min and then quickly cooled on ice. Two microliter of each mixture was loaded on a 5% polyacrylamide gel (ratio of acrylamide/bisacrylamide, 19 : 1) containing 50 m*M* Tris-borate (pH 8.3), 4 m*M* EDTA, and 10% glycerol. Electrophoresis was carried out with a sequencing-gel apparatus at 1200 V for 3–4 h in a cold room (4°C). After electrophoresis, gels were dried and subjected to autoradiography for 3–6 h at −70°C.

Using SSCP, we screened for mutations throughout the coding region (22 exons) of the *PTC* gene in our panel of esophageal and lung carcinomas. We detected aberrant SSCP patterns only in three esophageal cancers, within the polymerase chain reaction (PCR) products corresponding to exon 17 (Fig. 1). The PCR products were purified on agarose gels. DNA sequencing was performed with the AmpliTaq Cycle Sequencing Kit (Perkin Elmer, Foster City, CA) according to the manufacturer's protocol, using an ABI PRISM 377 DNA sequencer. Direct sequencing of the anomalous PCR products revealed a change from CTT to CTG at

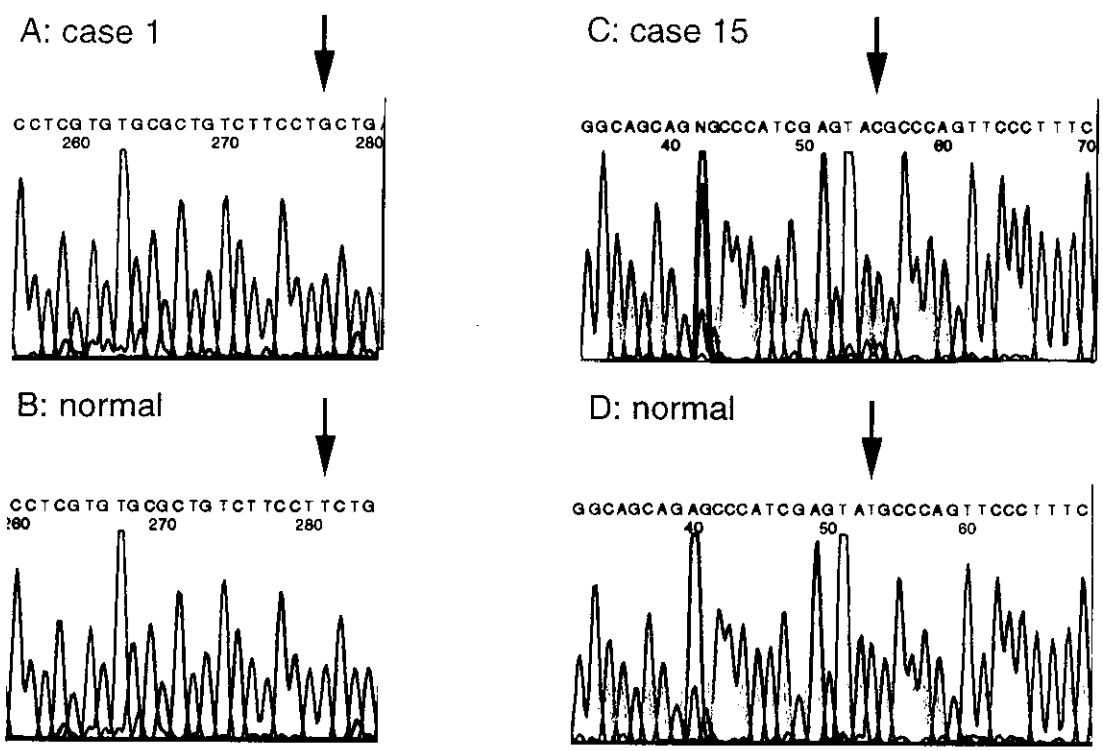

Fig. 2. Sequence analysis of PCR products corresponding to aberrant bands in Fig. 1. Nucleotide substitutions from T to G in case 4, and from T to C in case 15, are apparent.

codon 896 (Fig. 2, A and B) in all three tumors, but this alteration would not affect the amino acid sequence. We also noted a silent substitution of TAT for TAC at codon 820 (Fig. 2, C and D) in one of these three esophageal tumors. The results indicated that somatic alterations in *PTC* are not common in squamous cell carcinomas of the esophagus or lung.

Recent studies have revealed various genetic abnormalities in esophageal squamous cell carcinomas, including amplification of c-*myc*, *EGFR*, *int-2*, *hst-1*, and cyclin D genes,[11-14] as well as inactivation of *RB* and/or *p53*.[15-19] Since allelotype analyses have indicated high frequencies of allelic loss on chromosomal arms 3p, 9p, 9q and 17p in early-stage esophageal squamous cell carcinomas,[6,7] tumor suppressor gene(s) located in these chromosomal regions are likely to play significant roles in the carcinogenesis or progression of esophageal tumors. As allelic losses on 9q have also been reported in transient cell carcinomas of the bladder[20,21] and in squamous cell carcinomas of the lung and of the head and neck,[8,9] inactivation of a tumor suppressor gene on distal 9q may be important for carcinogenesis of squamous cell carcinomas in various tissues.

We previously performed a detailed LOH analysis of esophageal cancers using microsatellite markers on distal 9q, and defined a commonly deleted region within an approximately 200-kb interval at 9q31-32.[22] Although the NBCCS locus had been assigned to chromosomal region 9q22.3, we considered the possibility that the *PTC* gene might be related to some esophageal and lung squamous carcinomas, because the frequency of LOH in esophageal carcinoma in the more proximal region was as high as 41% and some tumors showed LOH at this region without having LOH at 9q31-32.[6] This result implied the possible involvment of another tumor suppressor gene(s) at a region more proximal to the commonly deleted region (9q31-32). However, the results presented here indicate that the *PTC* gene is unlikely to play any role in squamous cell carcinomas of the esophagus and lung, and that a different tumor suppressor gene in the region is related to carcinogenesis of these tissues.

Multiple self-healing squamous epitheliomata (MSSE) is an autosomal dominant disease characterized by invasive but self-healing skin tumors which are pathologically similar to well differentiated squamous cell carcinomas. Multipoint linkage analysis and haplotype studies have mapped the gene responsible for MSSE (ESS1) to 9q22-q31.[23] It is possible that the MSSE gene, if it should prove to be other than *PTC*, may play some role in squamous cell carcinogenesis.

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