DETAILED DELETION MAPPING OF CHROMOSOME 9p21-22 IN NASOPHARYNGEAL CARCINOMA

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ABSTRACT

Objective: To further refine the extent of deletion on chromosome 9p21–22 in nasopharyngeal carcinoma (NPC) and provide evidence for discovering new tumor suppressor gene. Methods: Loss of heterozygosity (LOH) on chromosome 9p21–22 was analyzed in 25 paired blood and tumor samples by using 11 high-density microsatellite polymorphic markers. Results: 17 of 25 cases (68.0%) showed LOH at one or more loci. Higher frequencies of LOH were found at four loci: D9S161 (35.0%), D9S1678 (31.5%), D9S263 (33.3%) and D9S1853 (33.3%), where 6 cases had a contiguous stretch of allelic loss. Conclusion: The minimal common region of deletion might be defined between D9S161 and D9S1853 (estimated about 2.7 cM in extent) at 9p21.1, suggesting that inactivation of one or more tumor suppressor genes located in this region may be an important step in NPC.

Key words: Nasopharyngeal carcinoma, Chromosome 9p21–22, Loss of heterozygosity, Tumor suppressor gene

Previous studies have showed that Epstein-Barr virus (EBV) infection, certain environmental factors and genetic factors were found to be closely associated with nasopharyngeal carcinoma. The rates of NPC in southern China and Southeast Asia are 25 times higher than that of in western countries. The statistic analysis revealed 5%–10% NPC patients have family history, there, genetic susceptibility might be an important factor in the pathogenesis of NPC. Unfortunately the alterations of common tumor suppressor genes are rare in NPC. Previous cytogenetic and molecular genetic studies have showed that loss of heterozygosity (LOH) on chromosome 9p21–22 is one of the most frequent genetic alterations in NPC and in many common sporadic cancers which suggested that there may be more than one TSG on 9p21–22. The p16/MTS1 gene located at 9p21.has been considered a putative tumor suppressor gene at this region. Although homozygous deletion and point mutations of p16/MTS1 occur frequently in a variety of human tumor cell lines, corresponding evidence of the same alternations in primary NPC samples is less common. To further precisely the refine extent of deletion on chromosome 9p21–22 in NPC, in this study, we performed loss of heterozygosity analysis on chromosome 9p21–22 in 25 paired blood and tumor samples using 11 high- density microsatellite polymorphic markers.

MATERRIALS AND METHODS

Tumor specimens

Twenty-five primary nasopharyngeal carcinoma biopsies and paired blood samples were collected from Xiangya Hospital and the Third Affinity of Hunan Medical University. None of the patients had received preoperative chemotherapy or radiotherapy. All tumors were undifferentiated NPC according to the World Health Organization (WHO) classification and clinical stages of those tumors were performed according to the UICC tumor-nodes-metastasis (TNM) classification.

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LOH Analysis

High molecular weight DNA was extracted from the tumors and the paired blood samples according to standard procedures. The concentration of DNA was diluted to 100 ng/μl and stored at 4℃. Eleven high-density polymorphic markers (located at 9p21–22) used in this study are listed along with their approximate chromosomal location in Table 1. All the primers used for the amplification of microsatellite markers were purchased from Research Genetics Inc. Polymerase chain reaction (PCR) reactions were performed in a total volume of 50 μl containing 200 ng of DNA, 0.2–0.3 μmol/L of each primer, 10 mmol/L Tris-Hcl, 50 mmol/L KCl, 15 mmol/LMgcl$_2$; 0.5 mmol/L of each deoxynucleotide triphosphate and 1.5–2.0 units of Taq DNA polymerase (SABC, China). The PCR was carried with an initial denaturation at 95℃ for 5 min, followed by 32 cycles at 94℃ for 50 sec, annealing temperature (52℃–58℃) varied according to the primer pairs used for 60 sec, 72℃ for 60 sec, and final extension at 72℃ for 10 min. 5–8 μl PCR products were diluted 1:1 with loading buffer (98% formamide. 0.1% xylene cyannolff, 0.1% bromophenol blue, and 10 mmol/L EDTA, pH 8.0) and denatured for 5 min at 95℃. Subsequently, 6–8 μl of this solution was electrophoresed in a 6%–8% polyacrylamide gel containing 8.0 M urea for 2–3 h at 550 V. After electrophoresis, the gel was stained in 0.2% $AgNO_3$ for 15 min and developed in a solution of 1.5% NaOH and 0.4% formalin for 5–8 min.

Definition of LOH

The signal intensities of alleles of tumor-tissue DNA were compared to those of normal-tissue DNA. LOH was defined as total loss of one allele or partial loss (50% or more reduction in one allele) compared to the retained one. Allele scorings were conducted independently by two investigators. Allelic imbalances were confirmed by a second PCR and a second electrophoresis run.

Table 1. Frequencies of allelic loss at 11 microsatellite markers on chromosome region 9p21–22 in NPC

Locus	Location	Genetic distance (cM)	LOH/informative cases (%)
D9S156	9p22	---	2/17 (11.7)
D9S162	9p22	3.8	2/18 (11.1)
D9S1870	9p21.3	3.2	3/18 (16.6)
D9S171	9p21.1	5.5	4/18 (22.2)
D9S169	9p21.1	6.2	4/17 (23.5)
D9S161	9p21.1	2.1	7/20 (35.0)
D9S1678	9p21.1	---	6/19 (31.5)
D9S263	9p21.1	0.8	6/18 (33.3)
D9S1853	9p21.1	1.9	7/21 ((33.3)
D9S165	9p21.1	4.3	3/18 (16.6)
D9S1874	9p13.1	2.6	1/17 (5.8)

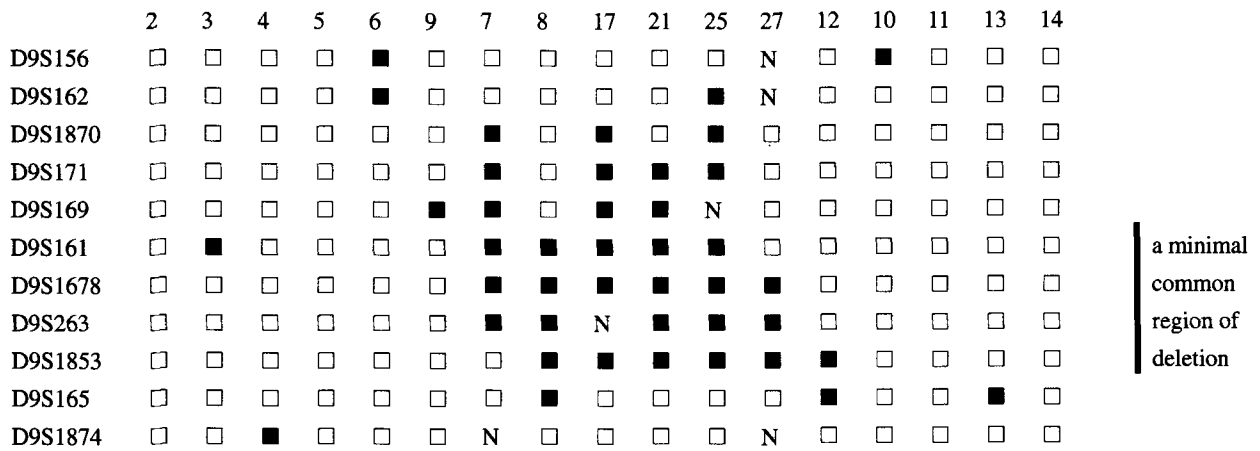
RESULTS

Of 25 NPC patients, 17 cases (68.0%) showed LOH at one or more microsatellite, 7 cases had contiguous allelic loss, Table 1 summarizes the frequency of allelic losses at the 11 high-density microsatellite markers. The highest frequency of LOH (35.0%, 7/20) was detected with D9S161 at 9p21.1; higher frequent allelic losses (31.3%–33.3%) were also found in three D9S161 adjacent loci: D9S1678, D9S263, D9S1853; but low frequency of p16/MTS-1 deletion (16.7%, 3/18) was observed by the adjacent D9S1870 marker; the lowest frequency of LOH (5.9%, 1/17) was detected with D9S1874 at 9p13.1. The results of LOH analyses of the 15 tumors that showed partial deletions involving 9p21–22 are summarized in Figure 1. Samples T-7, T-8, T-17, T-21, T-25, and T-27 had contiguous allelic loss from D9S161 to D9S1853, so a minimal common deletion region seems to lie between D9S161 and D9S1853 which genetic distances estimated about 2.7 cM at 9p21.1, approximately 14.0 cM to p16/MTS1 gene. In addition, associations between allele loss and clinical parameters, including patient age, tumor size, lymph node metastases were not statistically significant (P>0.05)

DISCUSSION

Cytogenetic and molecular genetic studies have shown that high frequencies of genetic alterations have been detected on 9p21–22 in numerous tumor types, including breast cancer, bladder tumors, lung tumors and melanomas (43%–57%). Specifically, LOH on 9p21–22

has been observed in 81% of head and neck squamous cell carcinomas. Allelic deletion in chromosome 9p21–22 in NPC has been studied previously. Huang et al. reported that 11 of 18 cases (61%) showed allelic loss on multiple loci of chromosome 9, and a common deletion region was D9S146 and D9S199 from 9q21–9p23, a homozygous deletion region lies between the loci D9S162 and D9S161 at 9p21–22 in one tumor was found. In our study, 25 primary undifferentiated NPC biopsies were examined for allelic loss at 11 loci of chromosome region 9p21–22. LOH was observed in 17 samples (68.0%). The number of NPCs (n=25) and the frequency (68%) of LOH are similar to Huang's results. But we have more precisely refined the location those deletions by using 11 high-density microsatellite markers for 9p21–22 region. A striking feature of our data was that a contiguous region of allelic loss from D9S161 to D9S1853 was observed in six NPC cases (T-7, T-8, T-12, T-17, T-21, T-25, T-27) and frequencies of LOH on these loci were higher than that of others, so a novel minimal common deletion region in 2.7 cM interval between D9S161 and D9S1853 at 9p21.1 was defined, approximately 14.0 cM to p16/MTS1 gene. The inhibitor of critical event in kinase 4 (CDKN2A/p16 and CDK2B/p15) have been mapped at to the region of 9p21. Loss of its function would be expected to uncontrolled cell growth. The high frequencies of CDKN2 alterations (heterozygosity/ homozygous deletion) have been reported in a variety of tumors, including breast cancer, lung cancer, bladder cancer and gliomas, which has raised the possibility that CDKN2 might be a candidate TGS in human malignancies. However, homozygous deletion and point mutation of the CDAN2 gene rarely been found in primary NPC. Furthermore, low frequencies of CDKN2 alterations, but frequent allele deletions for 9p21–22 markers have been reported in many types of tumors. The possible explanations are that another TSG (s) may be located with this region. In our panel of NPCs, the smallest common deletion did not involve microsatellite markers flanking CDK2. Our studies provide evidence that another gene in the D9S161–D9S1853 may be involved in the pathogenesis of NPC.



□: both alleles preserved; ■: LOH; N: no information

Fig. 1. Results of loss of heterozygosity at 11 microsatellite markers on chromosome region 9p21–22 in NPC

The human EST sequencing project have generated large numbers of partial gene sequences, some of these have been localized to specific chromosome bands. We obtained 25 3'ESTs mapped to D9S161–D9S1853 and invested their expression patter, found the EST (dbEST: 208825) was significant down-regulation or total absence in NPC. We cloned this novel gene full-length cDNA (Genbank accession No: AF149297). The functional characterization of this novel gene is currently under the way by cloning it into a mammalian expression vector and testing for its potential tumor suppression activity.

Furthermore, a minimal region of deletion around IFN gene cluster and MTAP gene mapped to 9p21–22 was observed in glioma, lung cancer and acute lymphoblastic leukemia, but had been eliminated as the potential target in melanomas. In our study, a minimal common region of deletion in NPC includes markers D9S161, D9S1678, D9S263 and D9S1853 but does not include the IFN gene cluster and MTAP gene locus, so our result also suggested that IFN gene and MTAP gene were unlikely involved in the development of NPC. In addition, associations between allele loss and clinical stages were not statistically significant ($P>0.05$) which suggest that inactivation of one or more putative tumor

suppressor genes located at this region may be an important step for NPC initiation and early development.

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