·英文论著·

鼻咽癌染色体3p14的精细等位基因缺失分析

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摘　要：目的　进一步明确鼻咽癌染色体3p14区域等位基因杂合性丢失(loss of heterozygosity, LOH)的频率与共同缺失区范围,以便分离该区域内与鼻咽癌相关的候选抑瘤基因。**方法**　选择位于3p14的6个高密度微卫星多态标记,对32例鼻咽癌组织进行LOH分析。**结果**　71.88%(23/32)的鼻咽癌在至少1个位点发生LOH,丢失频率较高的3个位点是D3S1313(46.43%)、D3S1300(50.0%)和D3S1312(44.44%)。在存在丢失的23例患者中,8例表现为一个连续的非随机的LOH区域,其最小共同缺失区为D3S1313～D3S1312(约3.4个厘摩)。且该区域的缺失与鼻咽癌临床分期、EB病毒感染有明显关系。**结论**　鼻咽癌在3p14的最小共同缺失区位于D3S1313～D3S1312之间,该区域可能存在一个尚未克隆的与鼻咽癌发生发展密切相关的抑瘤基因。

关　键　词:鼻咽肿瘤/遗传学;等位基因;基因,抑制,肿瘤;染色体3p14;杂合子检测
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Analysis of allelic loss on chromosome 3p14 in nasopharyngeal carcinoma

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Abstract: Objective　To further determine the frequency and common deletion region of allelic loss on chromosome 3p14 in nasopharyngeal carcinoma (NPC) in order to facilitate the isolation of the candidate tumor suppressor genes (TSGs) associated with NPC. **Methods**　Six high dense microsatellite polymorphic markers on 3p14 were selected to examine the loss of heterozygosity (LOH) in 32 NPCs. **Results**　Of the 32 specimens, 23 (71.9%) showed LOH in at least one locus. High frequencies of LOH were observed at loci D3S1313 (46.4%), D3S1300 (50.0%), and D3S1312(44.4%). Eight cases showed LOH in one continuous and nonrandom region between D3S1313 and D3S1312 (about 3.4 centimorgan). In addition, the LOH on 3p14 correlated with the tumor stage and Epstein-Barr virus (EBV) infection. **Conclusion**　A putative TSG involved in nasopharyngeal carcinogenesis may be present at the smallest common deletion region between D3S1313 and D3S1312.

Key words: Nasopharyngeal neoplasm/genet; Alleles; Genes, suppressor, tumor; Chromosome 3p14; Heterozygote detection

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Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with a high incidence and mortality in Southern China. Epidemiological studies have implicated that the pathogenesis of NPC correlated with

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Epstein-Barr virus (EBV) infection, certain environmental carcinogens, and some genetic factors[1].

Molecular genetic analyses have identified a frequent allelic loss of chromosome 3p in NPC. This suggests that the inactivation of putative tumor suppressor genes (TSGs) on 3p may be involved in the development of this cancer. Three distinct regions (3p13～14.3, 3p 14.3～21, 3p21～ter) were most frequent regions with loss of heterozygosity (LOH)[1]. In order to locate the TSG on 3p14 precisely, we used 6 high dense microsatellite polymorphic markers from 3p14 to determine the frequency and extent of 3p14 LOH in 32 NPC cases.

1 Materials and Methods

1.1 Patients and specimens

Primary NPC biopsies and corresponding blood samples were obtained from untreated patients collected at Xiangya Second Hospital, Central South University (Changsha, China). The data on the titers of Epstein－Barr Virus Capsid Antigen-IgA (EBV-CA-IgA) of each blood sample were obtained from the Department of Otolaryngology of Xiangya Second Hospital. All tumors were undifferentiated NPC according to the World Health Organization (WHO) classification. The clinical staging of the tumors was performed according to the tumor-nodes-metastasis (TNM) classification (UICC, 1997). Thirty-two tumor samples were determined by microscope to contain more than 70% malignant cells.

1.2 DNA extraction

High molecular weight DNA was extracted from the tumor and blood samples by conventional methods[2]. The concentration of DNA was diluted to 100 ng/μl and stored at 4℃.

1.3 Microsatellite analysis

Six microsatellite polymorphic markers (Table 1) on chromosome 3p14 were used to examine LOH by PCR analysis. The sequences of the primers and chromosomal localization were obtained with the Genome Database from the Internet. These primers were synthesized at the National Laboratory of Medical Genetics of Central South University (Changsha, China). PCRs were conducted in a DNA thermal cycler (Perkin Elmer), and contained 100 ng template DNA, 20 ng of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, and 1 unit of Taq DNA polymerase (Promega) in a final volume of 25μl. The PCR amplifications were performed as follows: initial denaturation at 94℃ for 5 min, followed by 28 cycles of denaturation at 94℃ for 30 s, annealing at the appropriate temperature for 30 s, with an extension at 72℃ for 40 s and a final extension at 72℃ for 5 min. The PCR products were then heat-denatured and electrophoresed on 8% or 10% denaturing polyacrylamide gel. The gel was stained in 0.2% $AgNO_3$ for 15 min and developed in a solution of 1.5% NaOH and 0.4% formaldehyde for 3～5 min. Allelic loss was inferred when the ratio of the two alleles in the tumor tissue was $<50\%$ of that seen in the matching normal tissue[3]. Constitutional homozygosity was regarded as uninformative. Samples showed LOH was identified by another independent amplification and electrophoresis.

Table 1 Frequencies of LOH at 3p14 in 32 NPCs

Markers	Location	Product Length(bp)	LOH/Informative Cases(%)
D3S3722	3p14.3	235	10/30(33.33)
D3S1547	3p14.3	96	7/27(25.93)
D3S1313	3p14.3	233	13/28(46.43)
D3S1300	3p14.2	236	13/26(50.0)
D3S1312	3p14.2～14.1	222	12/27(44.44)
D3S3631	3p14.1	227	0/29(0)

2 Results

Thirty-two matched tumor and normal DNA pairs were analyzed with six microsatellite polymorphic markers. LOH was observed in 23 of the 32 (71.88%) cases (Table 1 and Fig. 1). The remaining cases retained both alleles at all informative sites between D3S3722 and D3S3631. LOH was particularly frequent at D3S1313 (13/28, 46.43%), D3S1300 (13/26, 50.0%), and D3S1312 (12/27, 44.44%). However, LOH was not detected at D3S3631 in all cases. Of the 23 cases with allelic loss, five exhibited two or more LOH regions, and eight showed LOH in one continuous and nonrandom region between D3S1313 and D3S1312; six cases had LOH in only one locus, and the locus of LOH was located between D3S1313 and D3S1312 except in one case.

Table 2 shows the relationship between LOH on 3p14 and the clinical stage. Seven cases without LOH were staged as Ⅰ ~ Ⅱ, whereas only two cases were classified as Ⅲ. Of the 23 cases with LOH, 10 were staged as Ⅳ, 8 as Ⅲ, and 5 as Ⅱ. Furthermore, Table 2 shows the relationship between LOH on 3p14 and the titers of EBVCA-IgA. Of the 32 patients, 23 (71.9%) showed the antibody titers over 1:20. Of the 23 cases with LOH, 20 presented the titers of EBVCA-IgA over 1:20. Out of the 9 cases without LOH, only 3 showed the titers of EBVCA－IgA over 1:20.

Table 2 Correlation between LOH at 3p14 and NPC characteristics

	No. of Cases	LOH(+)	LOH(－)	P [3] Value
Total	32	23	9	
Clinical Stage				
Ⅰ/Ⅱ	12	5	7	=0.006
Ⅲ/Ⅳ	20	18	2	
EBVCA-IgA Titer				
(+)[1]	23	20	3	=0.006
(－)[2]	9	3	6	

注:1) EBVCA-IgA≥1:20; 2) EBVCA-IgA<1:20 or negative; 3) Fisher's exact test

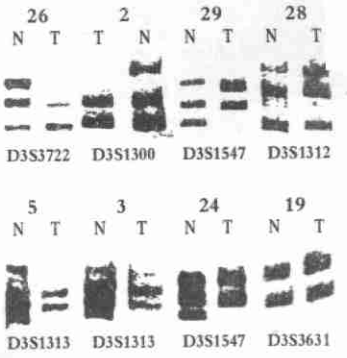

Fig 1 Representative examples of 3p14 LOH. Samples except case 19 showed LOH.
N: normal tissue; T: tumor tissue

3 Discussion

Tumor suppressor genes have been implicated in the development of several types of solid tumor and have been shown to be related to chromosomal rearrangements, particularly deletions[4]. The detection of LOH has been used to identify regions of the genome that may contain TSGs in various human cancers. LOH on chromosome 3p is one of the common genetic aberrations in many tumors[5]. Previous studies have demonstrated frequent allelic loss on chromosome 3p in NPC. The highest frequency of loss was found in the 3p13 14.3 region[1]. Hu et al[6]. reported 58% of allelic loss at D3S1217 (3p14.1 14.2). Multirangura et al[7]. showed that 75% LOH was observed at D3S1600 (3p14). A homozygous deletion was also found at 3p14.2 in two NPC cell lines[8]. These data strongly indicate that TSGs on 3p14 is involved in the development of NPC.

We have examined 32 NPC for LOH with 6 high dense microsatellite polymorphic loci at 3p14.1 14.3. Of the 32 tumors investigated, 23 (71.9%) were found to encounter allelic loss in at least one locus. The most frequent LOH occurred at D3S1300 (3p14.2). A continuous LOH region between D3S1313 and D3S1312 was observed in 8 cases. The smallest common deletion region contains the human common fragile site FRA3B and their genetic distance

is about 3.4 centimorgan.

The high frequency of allelic loss on 3p14.1 14.3 suggests that at least one TSG in this region may represent a critical event in the genesis of NPC. The fragile histidine triad (FHIT) gene, mapping at 3p14.2, was found to be LOH/deletion in more than 50% of digestive tract tumors[8]. However, some evidences against FHIT as a TSG were observed[9]. Although the FHIT gene is located within D3S1313～D3S1312, FHIT gene may be an innocent bystander in the human common fragile site[10]. No inactivating mutations in the FHIT gene were observed in primary NPCs, suggesting that it is unlikely to be involved in the development of NPC. These results indicate that an uncloning candidate TSG involved in nasopharyngeal carcinogensis may be located in this smallest common deletion region. This study provides a finer mapping of the TSG loci and facilitates the isolation of the gene.

A positive correlation between LOH on 3p14 and clinical stage has been previously reported in NPC[6], as the tumors in more advanced stages Ⅲ and Ⅳ showed a higher frequency of LOH than did tumors in stages Ⅰ and Ⅱ. Our data showed that the tumors staged as Ⅲ～Ⅳ were significantly ($P=0.006$) more likely to have LOH on 3p14 than the tumors staged as Ⅰ～Ⅱ, judged by the Fisher's exact test. In addition, we have identified a possible correlation between 3p14 LOH and EBV infection. The tumors with EBVCA－IgA titer≥1:20 were significantly ($P=0.006$) more likely to have LOH on 3p14 than tumors titer<1:20 or negative. A high incidence of 3p14 LOH in EBV-associated NPC has been reported[7]. Pathmanathan et al[11] have identified clonal proliferations of cells infected with EBV in preinvasive lesions related to NPC. Ohta et al[8] inferred that a possible role for EBV in the promotion of Chinese NPCs might be through viral DNA integration into the FRA3B region, which was suggested by the previous experiments of Rassool et al[12] showing apparent preferential integration of exogenous DNA into induced fragile sites in cultured cells. EBV associated with NPC might promote the induction of the FRA3B, contributing to the LOH/deletion on 3p14 in NPC and possibly to the inactivation of the putative TSG.

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