THE EXAMINATION OF AN EBV-DNASE GENE FRAGMENT IN THE PARAFFIN-EMBEDDED NPC, PRECANCEROUS AND FROM HIGH-RISK POPULATION NASOPHARYNGEAL TISSUES

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ABSTRACT

Objective: To examine if the EB virus appears in nasopharyngeal epithelial cells in the course of tumorigenesis in nasopharyngeal carcinoma (NPC). Methods: Based on the annual investigation of Sihui County, a cancer-prevention base of Sun Yat-sen University of Medical Sciences(SUMS) and Tumor Hospital out-patient clinic, more than 450 paraffin-embedded NPC tissue specimens, precancerous lesions and precancerosis from a high-risk population were collected for detection of an EBV-DNase gene fragment by an optimized PCR method. Results: In 145 of the 149 (97.3%) cases with invasive NPC, and 2 of 4 cases with *in situ* NPC, only 2 of the 155 subjects with precancerosis was DNase gene positive, and all 47 cases examined in the out-patient department of the Tumor Hospital with precancerous lesions in the nasopharynx were negative. Conclusion: The universal presence of an EBV-DNase gene in NPC tissues and the paucity of EBV-DNase gene in nonmalignant and precancerous lesions in the nasopharynx in a high-risk population imply that carcinogenesis of NPC occurs before the appearance of EBV in the nasopharynx.

Key words: Nasopharyngeal carcinoma, Precancerosis, EBV-DNAse gene, Polymerase chain reaction.

There is a long term in NPC tumorigenesis, including the stages of precancerosis conditions and precancerous lesions.[1-3] In order to investigate in which stage of tumorigenesis Epstein-Barr virus begins to appear in the lesions, in 1994 more than 450 specimens of nasopharyngeal biopsies including NPC and noncancerous nasopharyngeal lesion specimens were collected from the out-patient clinic of the Tumor Hospital and the cancer-prevention base of Cancer Center under SUMS, where the prospective ten-year program (1986—1995) about the nature population at high-risk for NPC was being undertaken. These samples were subjected to PCR of EBV-DNase gene detection using new primers and optimized methods[4] and PCR products were sequenced to verify their specificity.

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MATERIALS AND METHODS

Cell Lines and Tissues

EBV-positive cell lines (B95—8) and EBV-negative cell lines (K562) were taken from the Cancer Institute, SUMS. Guangzhou. Paraffin-embedded nasopharyngeal biopsy tissues taken from NPC patients and individuals from the high-risk population were provided by the pathological department, the Tumor Hospital, SUMS.

PCR and DNA Sequencing

Primer pair flanking 29% of the complete EBV-DNase gene (408 bp) were designed according to the open reading frame, BGLF5 from Genbank VO1555 and synthesized by Protein and Nuclear Acid Research Department, Hainan Medical College. The respective sequences of the sense primer the antisense and primer I is 5'-CTGATGTGGGACATATTGCG-3' and primer II is 5'>CCTCTTGTAAAGCGCAG-TGT< 3'. The paraffin sections of tissues were de-waxed in xylene, and then washed in ethanol for dehydration. The above de-waxed sections were lysed in 100 μl of solution containing 100 μg/ml proteinase K at 56°C water bath overnight with gentle shaking, followed by the denaturation of proteinaseK at 98°C for 10 minutes, and subsequently centrifuged to

obtain the supernatant as PCR templates. The templates were denatured at 94°C for 5 minutes, followed by 30 cycles of temperature profile (denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 1 minute). Finally the reaction mixture was extended at 72°C for 7 minutes. The PCR products were applied to agarose gel electrophoresis for UV illumination detection.

PCR products were purified using DNA fragment purification kit from BioRad Corporation (USA). DNA sequencing was performed by 377 DNA sequencer (PE Corporation, USA) using the antisense primer I and DNA sequencing kit from PE Corporation, USA.

RESULTS

Detection of EBV-DNase Gene

The 408 bp DNA bands standing for EBV-DNase gene were detected in B95–8 cell line and the tissues with NPC, whereas no corresponding band was detected in K562 cell line and the population at high-risk (Figure 1). The result of DNA sequencing indicated that the sequences of PCR-amplifited DNA fragment was in accord with the open reading frame, BGLF5, in Genbank VOl555.

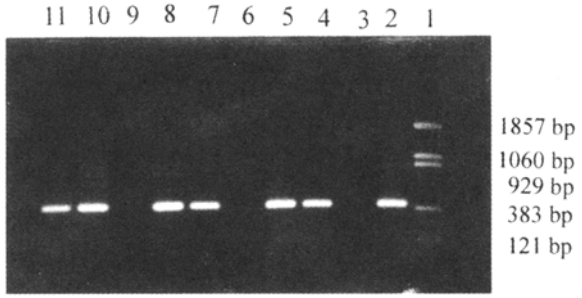

Fig. 1. PCR Detection of EBV-DNase Gene
1: Molecular Marker; 2: B95-8 Cell Line; 3: K562 Cell Line; 4,5,7,8,10,11: NPC Tissues; 6,9: Tissues of individuals from Population at High Risk.

PCR Detection of EBV-DNase Gene in Tissues with NPC and at High-Risk

The PCR results of the detection of EBV-DNase gene in NPC biopsies (confirmed pathologically) and in high-risk population were shown in Table 1.

Table 1. PCR Results of EBV-DNase Gene in NPC Cases and in Individuals of High-Risk Population

Group	No. of cases	No. of the positive	Percentage(%)
In situ NPC	4	2	
Invasive NPC	149	145	97.3*
High-risk population	155	2	1.3*

* $P<0.0005$, $X^2=303$ *as compared with the population at high- risk.*

Detection of EBV-DNase Gene in Precancerous Lesions of Different Types

No EBV-DNase gene was detected in 47 cases of clinically precancerous lesions in the nasopharynx, including 24 cases with low atypical hyperplasia, 14 medial atypical hyperplasia and 9 high atypical hyperplasia respectively. Meanwhile, the 34 precancerous lesions in the nasopharynx from the cancer-prevention base were examined for their EBV-DNase gene, consisting of 27 cases with low atypical hyperplasia, 6 medial atypical hyperplasia and 1 high atypical hyperplasia respectively. EBV-DNase gene was found in only 2 of the 27 low atypical hyperplasia biopsies. Altogether, EBV-DNase gene was detected in only 2 of the 81 (2.5%) cases of the precancerous lesions in the nasopharynx and there was no statistically significant difference among the positive rates in three kinds of the precancerous lesion ($P>0.05$).

The Relationships between EBV-DNase Gene and The Antibodies against EBV

The serological indexes as precancerosis were as follows: (1) VCA/IgA>1:80; (2) EDAb>60%; (3) Two or three of VCA/IgA, EA-IgA and EDAb positive; (4) One of VCA/IgA, EA/IgA and EDAb were is rising continuously. Precancerosis or precancerous lesions were referred to as the high-risk population with NPC. The PCR results of serologically positive "precancerosis" from the cancer-prevention base were shown in Table 2.

DISCUSSION

Many studies showed that EBV had close associations with NPC, but so far the etiological relationships between EBV and NPC have not been firmly proved. This project aimed to investigate the frequency of EBV-DNase gene distribution in various stages of nasopharyngeal tumorigenesis and then to

explore in which stage EBV begins to appear in the nasopharynx. The experimental results indicated that EBV-DNase gene was universally present in NPC tissues, but was rarely found in noncancerous nasopharyngeal tissues taken from individuals of the high-risk population. It suggested that EBV probably appeared in the nasopharyn geal epithelial cells after the carcinogenesis of NPC was accomplished.

Table 2. PCR Results of kinds of "precancerosis" from the cancer-prevention base

	VCA>1:80	EDAb** 60%	VCA>1:80 EDAb 30%	VCA>1:80 EA>1:5	EDAb 30% EA>1:5
No. of cases	89	60	61	10	9
No. of gene-positive	0	1	1	0	0
Gene-positive rate(%)	0.00*	1.7*	1.6*	0.00*	0.00*

* P>0.05 as compared with each other

** Antibodies against EBV-specific DNase

The 155 cases of "high-risk population" were screened, based on seriological information, from 40,000 nature population, more than 20,000 of whom had been followed for six years (since 1987). The PCR results of EBV-DNase gene distribution in "precancerous lesions" and "precancerosis" were almost identical. The results indicated further that the appearance of EBV-DNase gene in the noncancerous nasopharynx was rare in the tumorigenesis. The finding that only 2 out of the 4 cases of *in situ* NPC was positive with EBV-DNase gene was probably due to the fact that too few cancerous cells were left to be examined in the biopsy specimens after the bigger portion was used for pathological diagnosis. Yeung, et al.[5] also reported similar results that EBV gene was detected in 3 of the 6 cases with *in situ* NPC.

Recently, Sam, et al.[6] reported that EBV gene was detected in the nasopharyngeal tissues taken from high-risk population. Because of fewer cases examined and some suspicious individuals with NPC, Sam's results were somewhat incredible. On the contrary, in this project, as many as 155 cases from the nature population were collected in the cancer-prevention base supervised by SUMS, and sensitive PCR method was used for detection with DNA sequencing for its specificity verification. In addition, the paraffin-embedded specimens were chosen for examination. Therefore, our experimental results were more reliable in term of demonstrating the distribution of EBV-DNase gene in the tumorigenesis of the nasopharynx and might help to study the cause of NPC, to make differential diagnosis and other EBV-associated chronic or malignant diseases.

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