Detection of Epstein-Barr Virus DNA in Well and Poorly Differentiated Nasopharyngeal Carcinoma Cell Lines

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Abstract. Undifferentiated and poorly differentiated nasopharyngeal carcinoma (NPC) were known to be tightly associated with Epstein-Barr Virus (EBV). Its association with well differentiated NPC was also reported. In the present study, the presence of EBV was investigated by nucleic acid hybridization, Polymerase Chain Reaction (PCR), Immunoblot and in situ hybridization in two well differentiated NPC cell lines (CNE-1 and HK-1) and two other poorly differentiated NPC cell line (CNE-2 and CNE-3). Contrary to previous report indicating the absence of EBV in these cell lines, EBV DNA and proteins were present in all cell lines. The detection of EBV became more easily when the investigation was carried out on the nude mice tumor induced by transplantation of each NPC epithelial cell line. The EBV latent membrane protein (LMP1) was found by in situ hybridization to be integrated partly in the chromosomal DNA of these cell lines. The observations indicate that EBV could persist for a long time in the carcinoma cells established directly from well and poorly differentiated tumor biopsies and from transplantable NPC tumor in nude mice.

Keywords: Epstein-Barr Virus, Nasopharyngeal Carcinoma (NPC)

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most prevalent malignant tumors in Southern China and South Asia. The presence of EBV genomes not only in poorly and undifferentiated, but also well differentiated NPC carcinoma biopsies and the serological evidence of EBV in NPC patients indicate that the virus plays an important role in the development of NPC (1,2, 4–6). Three carcinoma cell lines were established directly from NPC biopsies in our laboratory, CNE-2 from poorly differentiated NPC, CNE-1 and HK-1 from well differentiated NPC, and another one CNE-3 cell line was established from transplantable poorly differentiated NPC tumor in nude mice. Among them, the presence of EBV genome in CNE-1, CNE-2 and HK-1 was not evident, since nucleic acid hybridization and anticomplement immunoenzymatic test done in these cell lines were revealed negative (7, 9–11). CNE-1 and CNE-2 were generally used as EBV negative epithelial cells so far (3). A possible explanation was the loss of EBV during a long culture in vitro or the absence of tight association of EBV with well differentiated NPC.

We asked whether these cell lines are really negative to EBV. Four methods such as PCR, immunoblot, in situ hybridization and Southern blot were used to search for the presence of EBV. The data reported here showed that some of established cell lines contained both EBNA-1 and LMP-1 genes and expressed their proteins. Moreover EBV DNA or protein became easily
detectable in the tumors induced by these cell lines in nude mice. Not only well differentiated, but also poorly differentiated cell lines contained EBV DNA. These data confirmed earlier report an association of EBV in both well and poorly differentiated NPC biopsies (6). The NPC cell lines established since 15-18 years and considered as EBV negative cells contained EBV DNA sequence and expressed two EBNA-I and LMP-1 latent proteins.

Materials and Methods

Cell Lines and Culture

Poorly differentiated human NPC cell line: CNE-2 (7) and two well differentiated NPC cell lines, CNE-1 and HK-1 (9,11) were established directly from NPC biopsies in our laboratory. Another poorly differentiated NPC cell line (CNE-3) was established from transplantable poorly differentiated NPC tumor in nude mice (8). They were maintained in RPMI-1640 medium supplemented with 20% foetal calf serum (FCS) at 37°C in 5% CO2. The RHEK-1 cell line, an immortalized human epithelial cell line kindly provided by Dr. Rhim (NIH, Washington), was grown in Dulbecco’s Modified Eagle’s Medium with 10% FCS and antibiotics.

Transplantation of NPC Cell Lines in Nude Mice

The cells growing exponentially were harvested and washed three times with fresh RPMI-1640 medium. The cell suspension (2 × 10⁶) was transplanted under the dorsal skin of the nude mice. The tumors with masses of 2-3 cm³ were obtained in about 30 days and they were frozen immediately at −160°C.

Southern Blot Hybridization

For Southern blot, the DNAs were extracted by standard methods; briefly, tumor tissues from nude mice were weighed, washed with phosphate saline buffered (PBS) and ground in a little basin with liquid nitrogen. 10 ml of TEN buffer (15 mM Tris-HCl, pH 8.0, 15 mM EDTA, 15 mM NaCl) was added per gram of tissue or 10⁸ cultured cells. 50 µg/ml of proteinase K (Boehringer Mannheim) and 1/20 (v/v) of 20% sodium dodecyl sulfate (SDS) were then added in samples and incubated for 4-5 hrs at 55°C followed by phenol-chloroform (V/V) extraction. 10 µg of the DNA precipitated by ethanol were suspended in TE buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA), digested with Xho-1 restriction enzyme (Sino-American Biothech. Co.) for 7 hrs at 37°C, electrophoresed through 0.8% agarose and transferred onto nitrocellulose membranes (Bio-Rad). Membranes were baked for 2 hrs at 80°C. The hybridization was done in 50 mM sodium phosphate (pH 7.2), 7% SDS, 1% BSA and 100 µg/ml denatured salmon sperm DNA, with about 10⁶ cpm/ml of probe at 65°C overnight. Membranes were then washed in 1 × SSC containing 0.1% SDS, and exposed to X-ray film at −70°C. Radiolabelled DNA probes were prepared with ³²P-dCTP (Amersham, England) by nick translation (specific activity = > 5 × 10⁷ cpm/µg DNA), using 3.1 Kb EBNA-1 and 2.9 Kb full length LMP-1 sequences prepared respectively from pBR322-W plasmid (a gift from Dr. H. Wolf, Germany) and pUC-ly plasmid (a gift from Dr. E. Kieff, Harvard Medical School, Boston) after BamH1 digestion.

Immunoblotting

Pelleted cells were suspended in protein extraction buffer (1% SDS, 1% β-mercaptoethanol, 1 mM PMSF, 20 mM Tris-HCl, pH 7.0) and sonicated twice for 3 min at 5Hz. Guanidine-HCl was then added to 5 mM and samples were centrifuged for 10 min at 5000 g. The supernatants were electrophoresed on a 12.5% polyacrylamide gel containing SDS and the proteins were transferred onto nitrocellulose filter. Then the filter was incubated overnight at 4°C with monoclonal anti-EBNA-1 and anti-LMP-1 antibodies (with 1: 50,000 dilution) as well as with sera from NPC patients. After washing, the filters were incubated for 1 hr at room temperature with peroxidase labelled anti-rabbit antibodies. A monoclonal antibody S12 directed against LMP-1 was from Dr. E. Kieff (Harvard Medical School, Boston). A monoclonal antibody against EBNA-1 was obtained in our laboratory.