ESTABLISHMENT OF A NOVEL CELL LINE (HNE\textsubscript{1}) DERIVED FROM A NASOPHARYNGEAL CARCINOMA

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A novel epithelial cell line, designated HNE\textsubscript{1}, was established from a biopsy specimen of a nasopharyngeal carcinoma (NPC). Electron microscopic examination of the HNE\textsubscript{1} cells demonstrated bi-directional differentiation, with some cells displaying features of poorly differentiated squamous cell carcinoma, while other cells appeared to have the morphology of poorly differentiated adenocarcinoma. The HNE\textsubscript{1} cell line has been passaged more than 100 times over a period of one year. We recently reported that the Epstein-Barr virus (EBV) nuclear antigen (EBNA) was detected in a low percentage of the HNE\textsubscript{1} cells examined at subcultures 5-8; the cells were also shown to be EBV DNA positive. Tumorigenicity of the HNE\textsubscript{1} cells was demonstrated by xenotransplantation in athymic nude mice. The developed tumors were characterized as well-differentiated squamous cell carcinomas upon histological examination. Karyotypic analysis of the HNE\textsubscript{1} cells demonstrated an aneuploidy with a modal chromosomal number of 71 at passage 5 and 101 at passage 20; 15 marker chromosomes were identified. The frequency of spontaneous sister chromatid exchange was found to be very high (87.6 ± 0.4/cell).

It has not been possible to establish a long-term EBV genome positive epithelial NPC cell line thus far. Often lymphoblastoid cell lines, not epithelial cell lines, were established from biopsy specimens of NPC. Only three permanent epithelial cell lines, CNE\textsubscript{1} and CNE\textsubscript{2} from NPC patients in China\textsuperscript{4,5} and NPC/HK\textsubscript{1} from a patient in Hong Kong\textsuperscript{6} have been successfully established. Unfortunately, none of these cell lines were confirmed to be EBV genome positive after long-term cultivation.

We have now been successful in obtaining an epithelial NPC tumor cell line, designated HNE\textsubscript{1}. In this report we present data on the establishment and properties of the HNE\textsubscript{1} cell line.

MATERIALS AND METHODS

Establishment of the HNE\textsubscript{1} Cell Line

A portion of NPC biopsy specimen (a poorly differentiated squamous cell carcinoma confirmed by histological examination) was immediately put in DF (DMEM-F\textsubscript{12}) medium containing penicillin 100 U/ml, gentamicin 100 μg/ml and kanamycin 100 μg/ml. The tissue was minced into fragments (0.5-1 mm\textsuperscript{3}); blood clots and necrotic tissue were teased away. The fragments were suspended in DF medium supplemented with 15% new-born calf serum, incubated for 16 hours at 4° C, washed with two changes of D-Hanks solution, digested with 0.15% trypsin for 10-15 minutes at 37° C, then rinsed twice with D-Hanks solution. The tissue fragments were seeded onto one side of a 25 ml glass tissue culture flask according to the method routinely used in our laboratory for the cultivation of human embryonic nasopharyngeal epithelial
A small volume of DF medium was added to the opposite side of the flask to maintain humidity. Several hours later, the flask was turned over to allow the medium to cover the adhered explants. The cells were incubated at 37° C. Half of the medium was changed three days later and the cells were then fed 2 times per week.

**Electron Microscopic and Histochemical Examination**

The HNE_1_ cells were assayed for lactic dehydrogenase and nonspecific acid esterase using previously published procedures. Cells for electron microscopic examination were rinsed twice with phosphate-buffered saline (PBS), detached by trypsin digestion or mechanical scraping, pelleted by low speed centrifugation, fixed in 3.5% phosphate-buffered glutaraldehyde and stored at 4° C. The cells were post-fixed with 1% buffered osmium tetroxide for one hour, dehydrated in acetone, and embedded in Epon 812 plastic. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined using an Hitachi 600 electron microscope.

**Detection of Epstein-Barr Virus Nuclear Antigen (EBNA)**

EBNA was examined according to the anti-complement immunofluorescence (ACIF) method of Reedman and Klein. FITC-lebeled anti-human C_3_ was produced by Cappel Corporation. Sera used in the experiment were patient's VCA-antibody positive and normal controls' fresh negative ones. Raji cells were included in each test as the positive control, and Ramos cells as the negative control.

**Chromosome Analysis**

Fifty-four hours after seeding of cells, colchicine was added (final concentration 1 ng/ml). Cells were harvested after incubating for 17 hours at 37° C. Metaphase chromosome preparations were prepared and G-banded. For sister chromatid exchange analysis, cells were treated with bromodeoxyuridine (BuDR), final concentration 10 μg/ml, after incubating for 27 hours; the cells were then incubated in the dark for an additional 54 hours, harvested and examined. The modal number of chromosomes was determined by counting 500 metaphases.

**Xenotransplantation**

BALB/c (nu/nu) mice of both sexes were purchased from the Shanghai Institute of Biological Products. HNE_1_ cells were washed twice with D-Hanks solution, then trypsinized with trypsin-versene. Detached cells (approximately 1.0×10^7 cells) in DF medium were inoculated subcutaneously in the subaxillary region of the nude mice. Histopathological examination was performed on tumors obtained 3 — 5 weeks later.

**RESULTS**

**Establishment of the HNE_1_ Cell Line**

Epithelioid cells interposed by a few fibroblasts grew as outgrowths of the small pieces of tissue after cultivation for 3 days. After growing to 90% confluence the cells were detached by trypsinization and mechanical means then transferred to another flask for subculture. The remaining attached cells in the flask were fed and kept for culturing. The cells could be subcultured repeatedly. Approximately 50% of the cells adhered to the flask after the first passage, and up to 90% of the cells adhered by the fifth passage. Generally, HNE_1_ cells grew steadily after cultivation in the flasks, seeding 6.5×10^5 cells/flask. The population doubling time calculated in the log phase of growth was 45.2 hours (data not shown). The cloning efficiency of HNE_1_ cells in soft agar was performed according to procedures previously published by us. Seeding 20000 cells/plate, the cloning efficiency averaged 32% counting 12 plates.

The histochemical staining of HNE_1_ cells