

Molecular pathological study of the human nasopharyngeal carcinoma CNE3 cell line

FEI LIU¹, WEI JIAO¹, XIANG-LAN MO², JIAO LAN¹, RUI-PING XIAO¹, XIANG-ZHEN ZHOU², ZHEN-LU HUANG², XIAO-MIN MO³ and GANG LI³

¹Research Center of Medical Sciences; ²Department of Pathology, The People's Hospital of Guangxi Zhuang Autonomous Region; ³Laboratory of Electron Microscopy, Guangxi Medical University, Nanning 530021, P.R. China

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Abstract. The present study aimed to identify the molecular pathological changes of the nasopharyngeal carcinoma (NPC) epithelial CNE3 cell line, which has been used in experimental studies for 20 years in a culture environment. The pathological type of NPC and the presence of the Epstein-Barr virus (EBV) were identified. CNE3 short tandem repeats (STRs) were amplified, analyzed and compared using metastatic carcinoma tissue from primary NPC. Immunohistochemistry (IHC) and *in situ* hybridization (ISH) were used to identify the immunophenotype and EBV-encoded small RNA (EBER) expression in nude mice transplanted CNE3 tumor cells. Polymerase chain reaction (PCR) and DNA sequencing were used to identify the EBV oncogene, *BamH1-A* right frame 1 (BARF1) and electron microscopy was used to analyze the organization of the ultrastructure. CNE3 was not cross-contaminated by other human cell lines and the EBV was no longer present in the CNE3 cells. The pathological type of CNE3 was transformed from an undifferentiated non-keratinizing carcinoma with focal adenocarcinoma differentiation into a poorly-differentiated adenocarcinoma. In conclusion, this knowledge on the molecular pathological changes of CNE3 may aid in the development of new research approaches for NPC.

Introduction

A study of nasopharyngeal carcinoma (NPC) recently demonstrated that the mortality rate of this disease was increasing in Guangxi, China (1). The current prevention and therapy for the disease does not indicate an optimistic outcome. Since CNE3 was established from a liver metastatic carcinoma

tissue of primary NPC (2), it has been used in basic studies of NPC (3-8). Certain studies revealed that the molecular biological characteristics were different between primary NPC and metastatic NPC, including expression of EBV-encoded small RNA 1 (EBER1) (9), zinc levels (10), karyotype and differentiation (11). Therefore, CNE3 may be useful for studies of metastatic NPC. However, the molecular pathology of CNE3 is altered due to long-term culture *in vitro*. The knowledge obtained from the continuing progress in molecular biological technology combined with the present study of the molecular pathology of CNE3 may provide useful data for subsequent studies.

Materials and methods

Cell culture. The human NPC epithelial cell lines, CNE1, CNE2, CNE3 and C666-1, were preserved in the Research Center of Medical Sciences, The People's Hospital of Guangxi Zhuang Autonomous Region (Nanning, China). As a control, CNE3 was obtained from the National Institute for Viral Disease Control and Prevention, Chinese Center of Disease Control and Prevention (Beijing, China).

Animal experiments. In order to establish the nude mouse tumor model of CNE3 through subcutaneous transplantation, Balb/c pure line mice were obtained from the Guangxi Medical University Laboratory Animal Centre (certification no. SCXK Gui 2009-0002). This study was approved by the ethics committee of The People's Hospital of Guangxi Zhuang Autonomous Region.

CNE3 short tandem repeat (STR) loci analyses. The CNE3 STR loci were authenticated using an ABI 3100 Genetic Analyzer (Microread Gene Technology, Beijing, China).

Histomorphology experiments. The tissues were obtained from a patient's primary nasopharynx foci in 1982, the same patient's metastatic liver carcinoma of primary NPC in 1988 and nude mice transplanted tumor in 2012. The tissues were fixed using 10% formalin and paraffin embedding, then sliced and stained with hematoxylin and eosin (HE). An optical analysis was then performed. Subsequent to being double stained

Correspondence to: Dr Wei Jiao, Research Center of Medical Sciences, The People's Hospital of Guangxi Zhuang Autonomous Region, Guangxi Medical University, 6 Taoyuan Road, Nanning 530021, P.R. China
E-mail: gxjw2005@163.com

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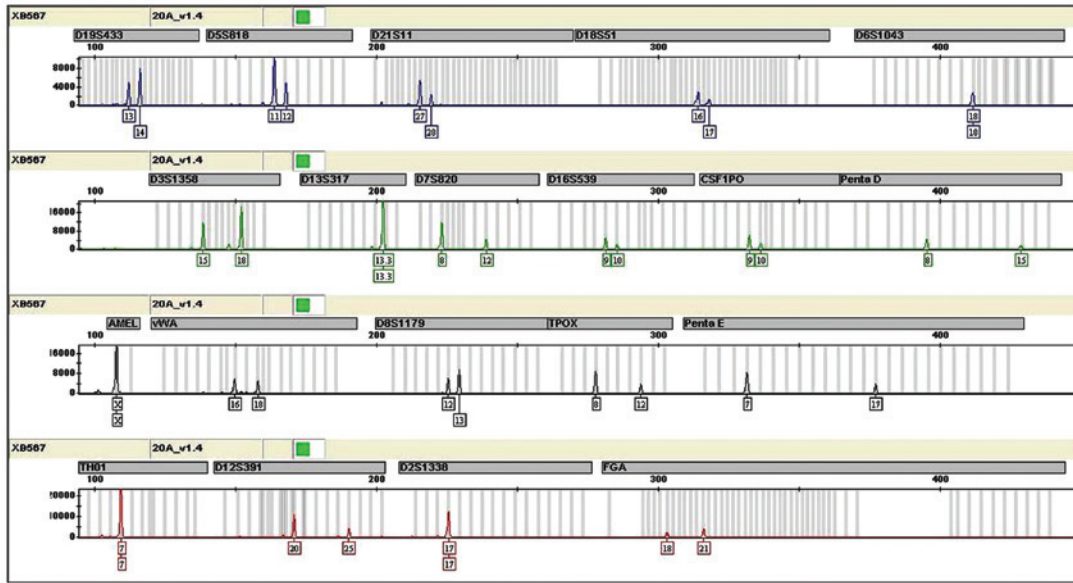


Figure 1. STR loci genotyping of CNE3. The 20 STR loci are not triallelic so the cell line is not cross-contaminated by other human cells. STR, short tandem repeat.

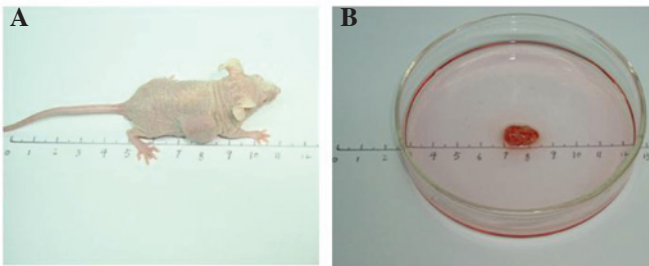


Figure 2. Balb/c nude mouse tumor model of CNE3. (A) A mouse with CNE3 tumor was measured by straight scale. (B) The tumor mass was measured by straight scale after the mouse was dissected.

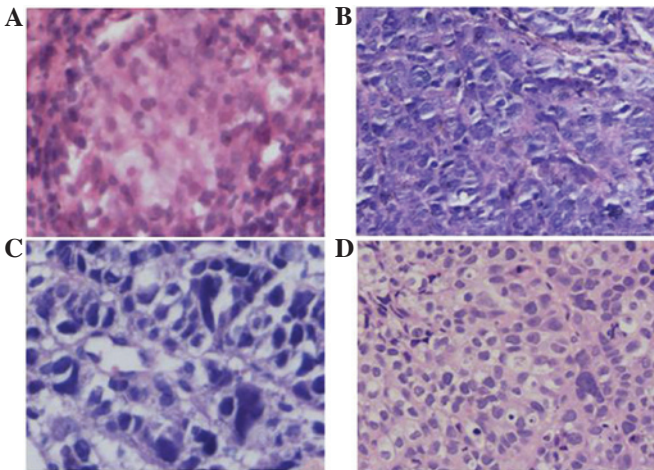


Figure 3. HE staining of NPC. (A) The carcinoma tissues of the primary nasopharynx foci were obtained from a patient in 1982 (x400). (B) Undifferentiated non-keratinizing carcinoma tissues of liver metastatic carcinoma of primary NPC were obtained from the same patient in 1988 (x200). (C) Primitive adenoid differentiation tissues of metastatic liver carcinoma of primary NPC were obtained from the same patient in 1988 (x400). (D) Nude mouse transplanted tumor tissues of CNE3 were obtained in 2012 (x200). HE, hematoxylin and eosin; NPC, nasopharyngeal carcinoma.

with uranyl acetate-lead citrate, the transplanted tumor was observed using a H-7650 transmission electron microscope (TEM; Hitachi, Tokyo, Japan).

Immunohistochemistry (IHC). A non-biotin horseradish peroxidase (HRP) ready-to-use two-step detection system (ZSGB-BIO, Beijing, China) and BX51 fluorescence microscopy (Olympus, Tokyo, Japan) were used in the IHC analysis. The positive brown granules, which were more abundant than the unspecific staining background, were mainly distributed in the cell nucleus (p63) or cytoplasm [cytokeratin (CK)5/6, CK7]. The positive cell rates and staining intensities were comprehensively analyzed in the intact slices using high power fields (x200 or x400). The results of the positive cell rates (<10%) and weak coloring were negative. The results of the positive cell rates (>10%) and dark brown granules were positive.

EBER *in situ* hybridization (EBER-ISH). An EBER-ISH kit (ZSGB-BIO) identified that the positive brown granules were mainly distributed in cell nuclei.

DNA extraction and polymerase chain reaction (PCR). DNA was extracted using the Genomic DNA Purification kit (Promega, Madison, WI, USA). The following primer sequences were used for the amplification of *Bam*H1-A right frame 1 (BARF1; NC_007605.1): BARF1 forward, 5'-CCAGGCTGTCACCGCTTTC-3' and reverse, 5'-CGCCAT TTGCCGAGTT-3'. The sequence length was 469 bp. The reaction conditions consisted of 12 μ l 2XTaq PCR Mix (Tiangen, Beijing, China), 0.5 μ l template, 0.5 μ l forward primer, 0.5 μ l reverse primer and 11.5 μ l ddH₂O. The reaction program consisted of an initial denaturation step at 95°C for 10 min, denaturation at 94°C for 35 sec, annealing at 57°C for 35 sec, extension at 72°C for 35 sec for 40 cycles and a final extension at 72°C for 10 min. The sequence was amplified using S1000 Thermal Cycler PCR (Bio-Rad, Hercules, CA, USA).

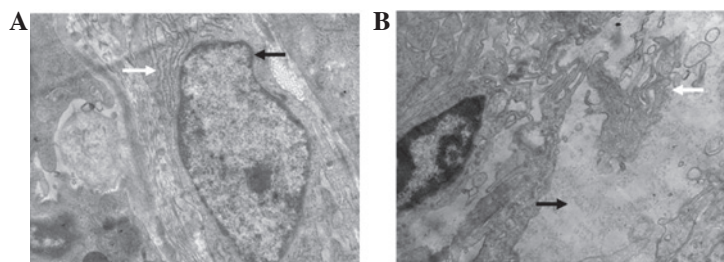


Figure 4. TEM observation of nude mouse transplanted CNE3 tumor tissues. (A) Abnormal nuclei (black arrow) and abundant rough endoplasmic reticulum ranged lamellar structure (white arrow; magnification, x20,000). (B) Microgranular cavities (black arrow) and microvilli on the surface of cavities (white arrow; magnification, x40,000). TEM, transmission electron microscopy.

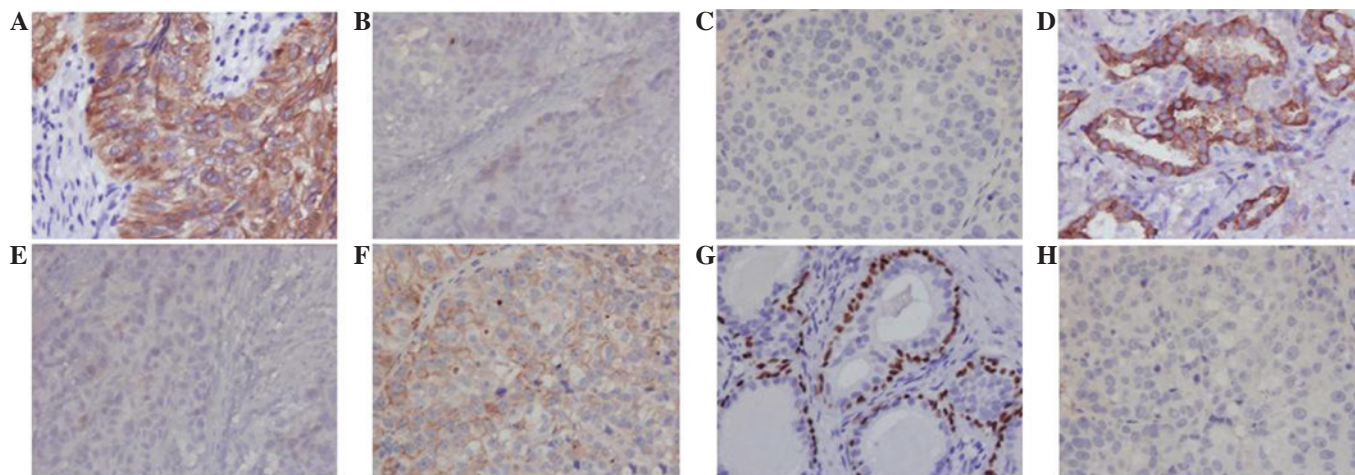


Figure 5. IHC expression of CK5/6, CK7 and p63 in various NPC tissues. (A) CK5/6 expression of squamous epithelial tissues were used as a positive control (x200). (B) CK5/6 was positively expressed in some of the metastatic liver carcinoma tissues of primary NPC (x200). (C) CK5/6 was negative in the nude mouse transplanted CNE3 tumor tissues (x200). (D) CK7 expression from lung adenocarcinoma tissues was used as a positive control (x200). (E) CK7 was positively expressed in some of metastatic liver carcinoma tissues of primary NPC (x200). (F) CK7 was positively expressed in the nude mice transplanted CNE3 tumor tissues (x200). (G) p63 expression from prostatic basal tissues was used as a positive control (x200). (H) p63 expression was absent in the nude mouse transplanted CNE3 tumor tissues (x200). IHC, immunohistochemistry; CK, cytokeratin; NPC, nasopharyngeal carcinoma.

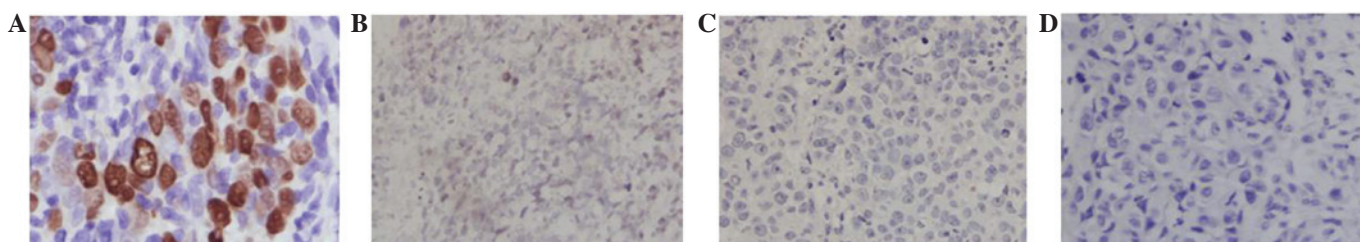


Figure 6. ISH expression of EBER in various NPC cells. (A) EBER of nasopharynx undifferentiated non-keratinizing carcinoma cells was used as a positive control (x200). (B) EBER was positive in some of metastatic liver carcinoma cells of primary NPC (x200). (C) EBER was negative in the nude mouse transplanted CNE3 tumor cells (x200). (D) EBER was negative in the nude mouse transplanted CNE3 tumor cells from Beijing, China (x200). ISH, *in situ* hybridization; EBER, Epstein-Barr virus (EBV)-encoded RNA; NPC, nasopharyngeal carcinoma.

Sequencing. Purified PCR products were analyzed by the 3730 automatic DNA sequencer (ABI, USA).

Results

Contamination status of CNE3. A total of 20 STR loci were not triallelic and the results revealed that CNE3 was not cross-contaminated by other human cells (Fig. 1).

Nude mouse transplanted tumor model. The transplanted CNE3 tumor volume was 0.15 cm³ after 14 days (Fig. 2).

Adenocarcinoma morphological characteristics. Microscopically, the cancer cells from the primary nasopharynx foci indicated the structure of an undifferentiated non-keratinizing carcinoma. The cells were polygonal, weakly basophilic, contained a large nucleus with prominent nucleoli, had little cytoplasm and an unclear cell boundary (Fig. 3A), which were arranged in sheets and nests. The cells of the primary metastatic liver carcinoma revealed a primitive adenoid structure. The cells had a circular form, rich cytoplasm and clear cell boundaries (Fig. 3B and C). The cells of the nude mice with the transplanted tumors indicated

EBNA1 is a unique viral protein that is found in the four forms of latent infection by EBV. It provides a distinct episome maintenance function by binding to oriP, which is the latent origin of DNA replication (17,18). Therefore, the expression level of EBNA1 is a key factor that episomes maintain in a steady state *in vitro*. If all episomes are lost, continuously mutated or partially missed, EBV will be lost.

The characteristics of CNE3 were studied and the pathological type was confirmed to be a poorly-differentiated adenocarcinoma with a low incidence rate. In conclusion, this knowledge on the molecular pathological changes of CNE3 may aid in the development of new research approaches for NPC.

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References

- Huang TR, Zhang SW, Chen WQ, Deng W, Zhang CY, Zhou XJ and Zhai RH: Trends in nasopharyngeal carcinoma mortality in China, 1973-2005. *Asian Pac J Cancer Prev* 13: 2495-2502, 2012.
- Jiao W: Establishment of a human epithelial cell line of nasopharyngeal carcinoma - CNE3 and its biological characteristics. *Journal of Guangxi Medical University* 12: 187-190, 1995 (In Chinese).
- Chen W, Lee Y, Wang H, Yu GG, Jiao W, Zhou W and Zeng Y: Suppression of human nasopharyngeal carcinoma cell growth in nude mice by the wild-type p53 gene. *J Cancer Res Clin Oncol* 119: 46-48, 1992.
- Teng ZP, Ooka T, Huang DP and Zeng Y: Detection of Epstein-Barr virus DNA in well and poorly differentiated nasopharyngeal carcinoma cell lines. *Virus Genes* 13: 53-60, 1996.
- Xia Y, Wong NS, Fona WF and Tideman H: Upregulation of GADD153 expression in the apoptotic signaling of N-(4-hydroxyphenyl)retinamide (4HPR). *Int J Cancer* 102: 7-14, 2002.
- Peng LX, Chen JX, Cheng JJ, Liu F, *et al*: Establishment of radio-resistant subline from human nasopharyngeal carcinoma cell line by repeating irradiation. *Chin J New Clin Med* 5: 1107-1109, 2012 (In Chinese).
- Yang XL, Liu XC, Huang L, Lan J, Zhang HY, Qin MB, Zhong YY and Mo ZN: Effect of TSA on nasopharyngeal carcinoma CNE3 cells and its mechanism. *Chin J Public Health* 26: 1029-1030, 2010 (In Chinese).
- Liu XC, Lan J, Nong CZ, Pan LL and Jiao W: Effects of mangiferin on induction of apoptosis and intracellular Ca²⁺ concentration in nasopharyngeal carcinoma CNE3 cells. *Chin J New Clin Med* 3: 805-807, 2010 (In Chinese).
- Tsai ST, Jin YT and Su IJ: Expression of EBER1 in primary and metastatic nasopharyngeal carcinoma tissues using in situ hybridization. A correlation with WHO histologic subtypes. *Cancer* 77: 231-236, 1996.
- Bay B, Chan Y, Fong C and Leong H: Differential cellular zinc levels in metastatic and primary nasopharyngeal carcinoma. *Int J Oncol* 11: 745-748, 1997.
- Lin JJ, He SY, Hao XP and Zong YS: Study on the primary and metastatic tumours of nasopharyngeal carcinoma using micro-spectrometry and immunohistochemistry. *Chin J Cancer* 17: 90-92, 1998 (In Chinese).
- Kaufmann O, Fietze E, Mengs J and Dietel M: Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. *Am J Clin Pathol* 116: 823-830, 2001.
- Mitroi M, Căpitănescu A, Georgescu CV, Mogoantă CA, Popescu C, Georgescu M, Mitroi G and Ioniță E: Expression pattern of CK7 and CK20 in nasal polyps, at patients with chronic rhinosinusitis with nasal polyposis. *Rom J Morphol Embryol* 52 (3 Suppl): S1051-S1057, 2011.
- Kuo TT, Shih LY and Tsang NM: Nasal NK/T cell lymphoma in Taiwan: a clinicopathologic study of 22 cases, with analysis of histologic subtypes, Epstein-Barr virus LMP-1 gene association, and treatment modalities. *Int J Surg Pathol* 12: 375-387, 2004.
- Cheung ST, Huang DP, Hui AB, Lo KW, Tsang YS, Wong N, Whitney BM and Lee JC: Nasopharyngeal carcinoma cell line (C666-1) consistently harbouring Epstein-Barr virus. *Int J Cancer* 83: 121-126, 1999.
- de Turenne-Tessier M and Ooka T: Post-translational modifications of Epstein-Barr virus BARF1 oncogene-encoded polypeptide. *J Gen Virol* 88: 2656-2661, 2007.
- Dittmer DP, Hilscher CJ, Gulley ML, Yang EV, Chen M and Glaser R: Multiple pathways for Epstein-Barr virus episome loss from nasopharyngeal carcinoma. *Int J Cancer* 123: 2105-2112, 2008.
- Sivachandran N, Thawe NN and Frappier L: Epstein-Barr virus nuclear antigen 1 replication and segregation functions in nasopharyngeal carcinoma cell lines. *J Virol* 85: 10425-10430, 2011.