2008

Association of Epstein-Barr virus with nasopharyngeal carcinoma and current status of development of cancer-derived cell lines

Charles A. Gullo PhD
Marshall University, gullo@marshall.edu

Wong Kein Low

Gerrard Teoh

Recommended Citation
Gullo C, Low WK, Teoh G. Association of Epstein-Barr virus with nasopharyngeal carcinoma and current status of development of cancer-derived cell lines. Ann Acad Med, Singapore. 2008;37(9):769–77.

Review Article

Association of Epstein-Barr Virus with Nasopharyngeal Carcinoma and Current Status of Development of Cancer-derived Cell Lines

Charles Gullo,[1] *BS, PhD*, Wong Kein Low,[2] *MBBS, MMed, FAMS*, Gerrard Teoh,[3,4] *MBBS, MMed, FAMS*

Abstract

It is well known that the Epstein-Barr virus (EBV) contributes directly to tumourigenesis in nasopharyngeal carcinoma (NPC), primarily in the undifferentiated form of NPC (WHO type III; UNPC or UC), which is commonly found in South East Asia. Unfortunately, research in NPC has been severely hampered by the lack of authentic EBV-positive (EBV+) human NPC cell lines for study. Since 1975, there have been more than 20 reported NPC cell lines. However, many of these NPC-derived cell lines do not express EBV transcripts in long-term culture, and therefore that finding may dispute the fundamental theory of NPC carcinogenesis. In fact, currently only one EBV+ human NPC cell line (C-666) in long-term culture has been reported. Hence, most of the NPC cell lines may not be representative of the disease itself. In order to better understand and treat NPC, there is an urgent need to develop more EBV+ human NPC cell lines. In this review, we discuss the authenticity of existing NPC cell lines and the impact of our understanding of NPC biology on the treatment of the disease and the relationship of EBV to NPC in the context of cell lines.

Ann Acad Med Singapore 2008;37:769-77

Key words: Carcinogenesis, Cell culture, Epstein-Barr virus, Hayflick's limit

Introduction

Although nasopharyngeal carcinoma (NPC) has been reported in almost all parts of the world, most cases of NPC are found in South East Asia, Southern China (including Hong Kong), North Africa and in the Eskimo population of Alaska, USA.[1-3] For reasons that still remain unclear, the Chinese are more susceptible to NPC than other races.[4] Earlier studies have suggested that dietary habits could be major contributors to the pathogenic process. These include the ingestion of salted fish and preserved food.[5,6] High-risk areas include southern China, Tunisia and Greenland, where the diet contain large amounts of N-nitrosodimethyamine (NDMA), N-nitrospyrrolidene (NPYR) and N-nitrospiperidine (NPIP),[7] common ingredients found in salted fish and preserved food. Moreover, environmental factors may also contribute to the development of NPC. For example, exposure to smoke or chemical pollutants, including trace elements (e.g. nickel) have been reported to be associated with the development of NPC.[8] A recent study suggests that among occupational hazards such as exposure to smoke, construction, metal, wood dust, motor fuel and oil, paint and varnishes etc., only wood dust was statistically significant for the development of NPC.[9] Therefore, the development of NPC disease is multi-factorial with genetics, diet, and environmental exposure all playing large roles.

NPC Histological Subtypes

The World Health Organization (WHO) classifies NPC into 3 varieties. Type I or squamous cell carcinoma (SCC) is seen in 25% of cases of NPC. These tumours produce keratin and demonstrate the presence of intracellular bridges when observed under the electron microscope. Approximately 20% of NPCs are of the Type II or non-keratinising carcinoma (NKC) variety. Tumour cells of the NKC phenotype demonstrate a range of cellular morphologies, from mature to anaplastic cells. Finally, Type III or undifferentiated carcinoma (UC) NPC constitutes

[1] Cancer Immunology Laboratory, Department of Clinical Research
[2] Department of Otolaryngology
[3] Multiple Myeloma Research Laboratory (MMRL), Singapore Health Services (SingHealth) Research Facilities
[4] Department of Hematology
Singapore General Hospital, Singapore
Address for Correspondence: Dr Charles Gullo, Cancer Immunology Laboratory, SingHealth Research Facilities, 7 Hospital Drive, Block A #02-05, Singapore 169611.
Email: charles.gullo@sgh.com.sg

the bulk (55%) of the tumours seen in patients with NPC.[10] Since there is no uniform morphological characteristic of NPC-affected tissues, diagnosis of UC NPC is usually based on the location of the tumour in the nasopharynx and the presence of EBV transcripts in the tumour cells. The EBV genome is found in transformed epithelia cells but not in lymphocytes. Clonal EBV genome is present in the early preinvasive dysplastic lesion or carcinoma in situ; indicating that infection of EBV precedes the development of malignant invasive tumour.[11]

Geographical Distribution of NPC subtypes

The distribution of NPC subtypes in certain geographical areas of the world does not necessarily conform to the overall worldwide distribution of NPC subtypes according to the WHO classification above. For example, in the regions where NPC is most prevalent, i.e. South East Asia, Southern China and Hong Kong, there is a predilection for UC NPCs. For example, most (85%) NPCs found in Singaporean Chinese are classified as UCs; and the incidence is greater among childhood and adolescent patients.[12] In contrast, most NPCs in Western countries (e.g. USA) are SCCs and thought to be related to the use of tobacco.[13,14] These differences contest the applicability of Western data and studies on Eastern populations, and *vice versa*. Derivations of cell lines from Eastern populations are limited and should be established based on the above findings.

The role of EBV in the pathogenesis of NPC

EBV

Epstein and his group discovered novel viral particles within cultured cells from patients with Burkitts's lymphoma in 1964,[15] and 4 years later; this new virus was named EBV. EBV is a relatively large gamma herpesvirus, and its DNA is double stranded and approximately 172 kilobases (kb) in length.[16] Approximately 90% of the adult population throughout the world are EBV-positive by serology. Elevated titers of IgA antibody to EBV viral capsid antigen (VCA) are usually found in patients with NPC, therefore this method of measuring patients' EBV-specific IgA antibodies is useful in screening for early detection of NPC.[2] EBNA1 and EBERS are expressed in all EBV-positive cases of NPC and LMP1 is present in up to approximately 65% of cases.[17,18] In almost all cases of EBV infection, the oropharynx is the primary site of infection, as well as the site of viral replication. Epstein-Barr virus is known to target primarily B-lymphocytes. *In vitro* studies demonstrate that EBV infects and potentially activates B cells by binding to the type 2 complement receptor (CR2, or CD21), the putative EBV receptor.[16] Hence, EBV appears to home to the oropharynx, and more specifically, the B cells within the oropharynx. The strain B95-8 can be found in EBV-positive cell lines such as Raji, Namalwa, and CA 46.[19] These cell lines are all of B-lymphocyte lineage. This strain has been used as a benchmark in order to check for EBV positively in NPC. The EBV latent proteins include the 6 nuclear antigens (EBNAs 1, 2, 3A, 3B and 3C, and EBNA-LP) and the 3 latent membrane proteins (LMPs 1, 2A, 2B). EBNA-LP is transcribed from variable numbers of repetitive exons. LMP2A and LMP2B are composed of multiple exons located on either side of the terminal repeats (TR) region, which is formed during the circularisation of the linear DNA to produce the viral episome. EBER1 and EBER2 are highly transcribed nonpolyadenylated RNAs and their transcription is a consistent feature of latent EBV infection.

EBV and NPC

It was in 1966 that Old et al first discovered the relationship between Epstein Barr virus and NPC, using *in situ* hybridisation and the anticomplement immunofluorescent (ACIF) assay.[20] Subsequent studies by others demonstrated the expression of EBV latent genes – Epstein-Barr virus nuclear antigen (EBNA), latent membrane protein-1 (LMP-1), LMP-2 and Epstein-Barr virus encoded small RNAs (EBER) – in NPC cells[16] confirming the infection of tumour cells by EBV. Intriguingly, expression of EBV early antigen (EA) is positively correlated with the consumption of salted and preserved food, suggesting that development of EBV-positive NPC could be related to dietary habits[21] and provides another link to the epidemiological studies with NPC. Future studies should consider the effects of dietary risk factors on the risk of specific histologic subsets of NPC, and not assume that the disease is aetiologically homogeneous. Since EBV infection indeed precedes clonal expansion of malignant cells,[22] EBV is thought to contribute, at least in part, to the overall pathogenesis of NPC. Many studies have shown that undifferentiated nasopharyngeal carcinomas (UNPC) are invariably EBV-positive, regardless of geographical origin.[13,23,24]

The process of EBV entry into keratinocytes and NPC cells is more complex, as both keratinocytes and NPC cells express only low levels of CR2 receptor.[25] In addition, the relevance of serological tests for EBV infection in predicting the occurrence of NPC is presently still unclear. Specifically, a positive serological test for EBV EA[26] may be found in greater than 80% of patients with NPC in Singapore,[27] yet many normal individuals also express a positive EBV EA serological test, and never develop NPC. The procedures that researchers have carried out, such as PCR, revealed expression of the LMP2A and LMP2B genes and of latent transcripts running through the BamHI A region of the EBV genome in the opposite direction to the conventional lytic cycle mRNAs transcribed over this region.[28,29] The

BamHI A transcripts have also been detected in other EBV associated tumours such as Burkitt's lymphoma and Hodgkin's lymphoma.[30,31] Analysis using western blot suggested a latent EBV infection in UNPC, and the expression of BZLF1 has been reported in some cases,[32] although the tumour cells do not seem to be fully permissive for virus replication. These findings are difficult to reconcile with the frequent detection of antibodies against structural viral proteins in the sera from patients with UNPC; especially patients with UNPC have raised IgA antibody titers to the VCA, EA, and MA complexes.[2] The rise in IgA titres to these antigens can be noticed before the development of UNPC and correlates with tumour burden, remission and recurrence.[33,34] Recently, a group in Taiwan used an animal model to carry out functional analysis of EBV in NPC cells. Their results showed that EBV-positive tumours grew faster than EBV-negative tumours, and also had clonal EBV terminal repeat sequences. Furthermore, EBV up regulates host genes only in cells that express those genes but not in cells that do not express them.[35] All these studies have unfortunately been greatly hampered by the lack of authentic EBV+ NPC cell lines, and little more is known about the mechanisms of entry of EBV, and the growth and survival NPC cells. In fact, numerous cell cultures that were initially positive for EBV eventually lost EBV expression after prolonged cell culture.

EBV Activation of Anti-apoptotic and Cell Proliferation Pathways in NPC

NPC is consistently associated with Epstein-Barr virus (EBV) infection. EBV-encoded LMP1, expressed in most of NPC, has been suggested to have an important role in the pathogenesis and development of NPC and its expression correlates with poor prognosis. LMP1 molecules aggregate in the cell membrane and through 2 C-terminal activating regions, interact with TNF receptor associated factors (TRAFs) and TNF receptor associated death domain protein (TRADD).[36-38] In vitro, LMP1 expression in epithelia cells can upregulate the expression of intercellular adhesion molecule 1 (ICAM-1), CD40, and cytokines such as interleukin 6 (IL-6) and IL-8.[39,40] LMP1 also can induce the expression of CD70 antigen, a member of the TNF family, in epithelial cells in vitro.[17,41] LMP1 can induce a matrix metalloproteinase, MM-9, through C-terminal activating regions 1 and 2 (CTAR-1 and CTAR-2), an effect blocked by overexpression of inhibitor of nuclear factor kappa B (IκB).[42] LMP1 encoded by EBV is a membrane protein that activates multiple signalling pathways and transcription factors, including Nuclear Factor-kappaB (NF-κB). NF-κB activation is necessary for Hodgkin/Reed-Sternberg (HRS) cells to proliferate and inhibit apoptosis.[43] Furthermore, activation of NF-κB is essential for B-cell immortalisation by EBV and LMP1-mediated transformation of fibroblasts.[44,45] EBER-negative Hodgkin Lymphoma (HL) also displays constitutive NF-κB activation, indicating that without LMP1, other mechanisms exist to activate NF-κB. LMP1 has been shown to activate the mitogen activated protein kinase pathways and Janus kinase (JAK) signal transducers and activators (STATs) of transcription pathway in epithelia cells, as well as in B cells.[46] LMP1 and 2A also activate the phosphatidylinositol 3'-OH kinase (P13K)/Akt pathway, which is commonly activated inappropriately in malignancy.[47] A recent study showed that P13K/Akt pathway was of importance in NPC pathogenesis. P13K/Akt pathway activation with subsequent phosphorylation and inactivation of GSK-3β and nuclear β-catenin accumulation were characteristic of primary NPC specimens.[47] Thus it is clear that NPC uses its viral proteins to activate numerous cellular pathways in the NPC tumour that results in proliferation and prevents the transformed cells from dying.

The B Specific Properties of the Viral Genes of EBV

Primary EBV infections occur early in life and in most individuals it is asymptotic. *In vitro*, EBV readily infects resting peripheral blood B cells that express the EBV receptor, CD21, resulting in latent infection and proliferation. The Lymphoblastoid cell line (LCL) is an in vitro model of transformed B cells (B cells that are infected by EBV). The LCL carries many copies of the EBV viral genes in an episomal fashion and constantly expresses a limited set of viral gene products consisting of latent proteins, (6 nuclear antigens; EBNAs 1, 2, 3A, 3B, 3C and –LP and 3 latent membrane proteins; LMPs 1, 2A and 2B).[45] LCLs expressed high level of B cell activation markers CD23, CD39, and CD70, and of the cellular adhesion molecules LFA1 (CD11a/18), LFA3 (CD558) and ICAM1 (CD54).[48] These markers are induced to high levels when the B cells are activated by antigenic or mitogenic stimulation, suggesting that EBV-induced immortalisation can be brought out through the constitutive activation of the similar cellular pathways that result in physiological B-cell proliferation.[49] The EBNA1 protein binds to viral DNA and allows the EBV genome to be maintained in the B cells as a circular DNA episome.[50] EBNA-2 upregulates the expression of LMP 1 and LMP2, as well as cellular proteins that contribute to the growth and transformation of B cells.[51] LMP-1, which acts as an oncogene,[52] mimics the constitutively active form of the B-cell surface molecule CD40 inducing a CD40 signalling response.[53] It also upregulates the expression of the antiapototic proteins (e.g. Bcl-2 and A20)[54] and results in cytokine production (e.g. IL6 and IL8).[40,55] Thus, viral proteins confer strong pro-survival signal to EBV infected cells of the B cell lineage. LMP-1 also mimics the functions of the members of the tumour necrosis factor receptor

(TNFR) family, activating a number of signalling pathways in a ligand independent manner.[56] Many of these downstream pathways result from the ability of TNFR- associated factors (TRAFs) to interact either directly with CTAR1 or indirectly via the death domain protein TRADD to CTAR2.[45] The binding of TRAFs to the cytoplasmic tails of LMP1 provides a platform for the assembly and activation of upstream signalling molecules including non-receptor tyrosine kinases.[57,58] However, since NPC is a result of infection of the epithelia, EBV LCL biology may not be representative of the Biology of EBV in NPC itself.

Thus, Epstein-Barr virus (EBV) infection in vitro causes transformation of B cells and can result in the generation of B lymphoblastoid cell lines. These cells have been useful as tools for 1) raising cell-mediated immunity (both CD4+ and CD8+ T cells) against EBV, 2) diagnostics of genetic metabolic disorders, 3) understanding EBV pathogenesis and 4) interrogating B cell biology (e.g. CD40 and CD40L signalling). Although there are many similarities between EBV/LCL and EBV/NPC tissue or cell lines there are some notable differences. One important difference is that whereas LCLs constitutive express of a small number of EBV-coded latent proteins, the nuclear antigens EBNA 1, EBNA 2, EBNA 3 and EBNA-LP and the latent membrane protein (LMP), tissue biopsies from EBV/NPC shows no expression of EBNA 2, EBNA 3 or EBNA-LP.[18] Furthermore, none of the biopsies studied expressed any of the EBV lytic cycle antigens. Another major difference is the cell of origin for EBV infection. The strongest links between the association of EBV and human cancer is the undifferentiated nasopharyngeal carcinoma which is of epithelial origin while LCLs are universally B cells. The tissue tropism is not defined by the receptor for EBV as CR2 is also believed to be the EBV receptor for B cells as well.[59] However, the immune properties such as CD40-CD40L interactions as well as the mode of EBV viral gene maintenance in the B cells are likely to play significant roles in the observed differences between epithelial cell EBV/NPC and B cells EBV/LCL EBV pathogenesis.

Authentic EBV+ Human NPC Cell Lines

Today there are only limited numbers of authentic EBV-positive NPC cell lines in existence. HONE-1 C40 (subclone of HONE-1),[60,61] which has maintained EBV-positively up to 42 passages and contains 85% to 90% EBV nuclear antigen (EBNA)-positive cells may be an example of an authentic NPC cell line. Other examples include CG-1[62] and C-666[63] that have been shown to maintain EBV positively in long-term culture. The authenticity of these cell lines is complicated due to the fact that the EBV genome is lost in many cell lines after long-term culture.

Original Tissue Used for Cell Line Development

In general, tissues from 2 sites from NPC patients have been used establish long-term cell lines. Most of the cell lines have been derived from primary tumour tissue samples obtained from the nasopharynx, e.g. CNE-1,[1] CG-1,[62] and C15[64] (Table 1). A number of cell lines were also derived from metastatic NPC tissue from other sites. For example, the NPC-BM1 cell line was established from bone marrow biopsy sample in a patient with bony metastases and the C17 cell line was derived from a skin biopsy sample in a patient who had skin, bone and liver metastases and C18 cell line, from a cervical lymph node biopsy in a patient with locally advanced tumour with regional lymph node involvement.

Short-term EB positive NPC-derived Cell Lines

Several cell lines have been derived from UC and poorly differentiated SCC NPC tissue (the most common subtypes of NPC tissue found in the South East Asia, South China and Hong Kong) that were initially EBV-positive (Table 1). These include: UC derived C-15, C-17, C-18, C666-1 (HK666), CAO, CG 1, CNE-2, CNE-2Z, CNE-3, HNE-1, HONE-1, NPC/HK2117 (Xeno-1), NPC/HK1915 (Xeno-2), NPC/HK1530 (Xeno-3), NPC-TW03, NPC-TW 06, NPC-TW07, SUNE and SUNE.[60-76] Other cell lines were also derived from the less common EBV+ SCC NPC tissue, including NPC-TW 01 (a.k.a. NPC-TW 039), NPC-TW 05, and NPC-TY861.[70,71,77,78] The limitation of these cells lines are that they have become EBV-negative after prolonged culture, thus their physiological relevance is uncertain. By 1989, only 3 permanent epithelioid NPC cell lines were established, CG1 and CNE2 (from China), and NPC/HK1 (from Hong Kong). Again, none of these NPC cell lines contained the EBV genome despite extensive testing; further suggesting that either EBV infection was unrelated to the development of NPC; or that these cell lines were not representative of NPC tissue or both. CG-1 and CNE were both EBV-positive but after long-term culture they lost their EBV positivity.[79]

EBV Negative NPC-derived Cell Lines

In contrast to the initially EBV+ tumour tissue, several NPC-derived cell lines were generated from EBV- tumour tissue. These included those derived from well-differentiated SCCs – CNE, NPC/HK1, NPC-TW 02 (NPC-TW 076), and NPC-TW 08.[70,71,77,80-82] Second generation NPC-derived cell lines were occasionally produced from the parental cell line. For example, the CNE epithelioid NPC cell line subsequently produced the CNF fusiform cell line. Importantly, neither CNE nor CNF cell lines demonstrated the presence of EBV virus particles,[82] again challenging a causal relationship between EBV and NPC.

Attenuation of EBV Expression in Long-term NPC-derived Cell Lines

Expression of EBV DNA has been observed to become attenuated after longer-term culture in many cell lines, including NPC-TW 01, NPC-TW 03, NPC-TW 05, NPC-TW 06, and NPC-TW 07, etc. suggesting that EBV does not seem to be required for the survival and proliferation (Table 1).[60,71,81,82] In other words, EBV might facilitate the initiation of the pathological process that eventually leads to NPC, but is not required for subsequent growth of tumour cells *in vitro*. Currently, this issue cannot be resolved since there is a lack of EBV+ NPC cell lines that are truly

Table 1. Long-term Solid Tissue Cultures Derived from NPC Tumours

Cell line	NPC WHO classification	Derived cell type	Culture method	EBV status	References
C-15	NKC	epithelial/syncytial	subcutaneous xenografting /Swiss nude mice	IP	(64)
C-17*	NKC	epithelial/syncytial	subcutaneous xenografting /Swiss nude mice	IP	(64)
C-18†	NKC	epithelial/syncytial	subcutaneous xenografting /Swiss nude mice	IP	(64)
CAO	—	—	xenografting in nude mice	IP, LP	(68)
X666	UC	epithelial/syncytial	subcutaneous xenografting a in BALB/c (*nu*/*nu*) athymic nude mice	IP, LP	(90)
C666	UC	epithelial/syncytial	RPMI 1640, 1% fetal bovine serum (FBS)	IP, LU	(67,90)
C666-1; HK666	UC	epithelial/syncytial	RPMI 1640, 1% FBS	IP, LP	(63,67)
CG1	NKC	epithelia	DMEM/F12, 5% FBS	IP	(62)
CNE-2	UC	epithelial	RPMI 1640, 10% FBS	IP	(72)
CNE-2Z	UC	epithelial	RPMI 1640, 10% FBS	IN	(76)
CNE-3	UC	epithelial	RPMI 1640, 10% FBS	IN	(65)
HNE-1	UC	epithelial	DMEM/F12	IP, LN	(60,61,74)
HONE-1	UC	epithelial	RPMI 1640	IP, LN	(60,61,74)
NPC/HK1530; Xeno-3	UC	epithelial	subcutaneous xenografting in athymic mice	IP, LN	(69)
NPC/HK1915; Xeno-2	UC	epithelial	subcutaneous xenografting in athymic mice	IP, LN	(69)
NPC/HK2117; Xeno-1	UC	epithelial	subcutaneous xenografting in athymic mice	IP, LN	(69)
NPC-TW 03	UC	epithelial	DMEM, 5% FBS	IP, LN	(70,71)
NPC-TW 06	UC	epithelial	DMEM, 5% FBS	IP, LN	(70,71)
NPC-TW 07	UC	epithelial	DMEM, 5% FBS	IP, LN	(70,71)
SUNE	UC	epithelial	RPMI 1640, 10% FBS	IP	(66)
SUNE1	UC	epithelial	DMEM, 5% FBS	UNK	(14)
NPC-TW 04	UC	epithelial	DMEM, 5% FBS	IN, LN	(70,71)
NPC-TW 09	UC	epithelial	DMEM, 5% FBS	IN, LN	(70,71)
CNE	SCC	epithelial	RPMI1640, 15% FBS	IN, LN	(80,82)
NPC/HK1	SCC	epithelial	RPMI1640, 15% FBS	IN, LN	(81)
NPC-TW 02; 076	SCC	epithelial	DMEM, 7% FBS	IN, LN	(71,77)
NPC-TW 08	UC	epithelial	DMEM, 5% FBS	IN, LN	(70,71)
NPC-TW 01; 039	SCC	epithelial	DMEM, 7% FBS	IP, LN	(71,77)
NPC-TW 05	UC	epithelial	DMEM, 5% FBS	IP, LN	(70,71)
NP69SV40T	SCC	epithelial	Immortalised by SV40T	IP, LP	(46)
NP39E6/E7	SCC	epithelial	Immortalised by HPV16E6/e7	IP, LP	(46)
NPC-BM1‡	UC	epithelial	RPMI1640, 10% FBS	UNK	(91)
NPC-F/L	UC	epithelial	EF-10, 10% FBS, co cultivated with EBV-transformed cell line.	IP, LP	(85)

IN: initially EBV-negative; IP: initially EBV-positive; LN: long-term culture EBV-negative; LP: long-term culture EBV-positive; LU: long-term culture EBV-status unknown; NKC: non-keratinising carcinoma; SCC: squamous cell carcinoma; UC: undifferentiated carcinoma; UNK: unknown.

* Derived from metastatic tissue in the skin

† Derived from metastatic tissue in the regional lymph node

‡ Derived from metastatic tissue in the bone marrow

representative of NPC itself.

Development of NPC-derived Cell Lines Using Xenografts

Several cell lines have been derived from transplantation of a human source to an animal one. NPC-derived cell lines; C15-C18 was subcutaneously xenografted into Swiss nude mice. X666 was derived from asubcutaneous xenograft of BALB/c (*nu/nu*) athymic nude mice. NPC/HK1530 (Xeno3), NPC/HK1915 (Xeno2) and NPC/HK2117 (Xeno3) were all derived from athymic murine xenografts. The CAO NPC cell line was an EBV-positive NPC-derived from a 54-year-old male patient from Shanghai China and was propagated in a BALB/c nude mouse.[83,84] Again, since these cell lines are not derived from primary cultures, the relationship to NPC itself is controversial.

Development of NPC-derived Cell Lines Using Other Methods

Currently there are hybrid tumour cells known as NPC-F/L that have been developed.[85] These were made using a co-culture system in which EBV transformed LCL of adenoid origin was cultured in the presence of NPC tissue. NPC-F/L contains some EBV genome such as the EBNA and EA but do not have VCA. In another method, the SCC derived EBV-positive cell epithelia squamous cell carcinoma NP39E6/E7 was generated by immortalisation with HPV16E6/E7 and NPC cell line NP69SV40T, was immortalised by SV40LT Ag.[46] These 2 methods, however, may not be truly representative of the biological properties of NPC, as they are not from pure primary culture.

Difficulties in Culturing NPC Cells *In vitro*

Limited Tissue Quantity from PNS Biopsies

There are many inherent limitations to culturing NPC cells *in vitro*. Typically, PNS biopsies provide only small tissue fragments. In order to increase the amount of available tissue, subcutaneous tumours may be first transferred to athymic mice for initial *in vivo* expansion before seeding into tissue culture flasks for *in vitro* culture.[63] Besides the potential introduction of murine cells, this method is likely to result in changes to the primary human biopsy tissue sample.

Contamination by Other Cell Types

It is common for biopsy tissues to be heavily infiltrated with lymphoid cells and cells of connective tissue origin. Hence, it is virtually inevitable that cell lines established from tumour explants acquire a lymphoblastoid phenotype during the initial stages of long-term cell culture.[67] Several investigators have used serum free medium (SFM) to inhibit the fibroblast/mesenchymal cell growth and promote tumour cell proliferation, e.g. the NPC-TW039 and NPC-TW076 cell lines.[77] Established NPC-derived cell lines were later converted from SFM to complete medium containing foetal bovine serum (FBS). This may also have a dual benefit of helping to decrease the serum dependent loss of EBV status.[77] Other investigators have used pipetting during cell culture to remove fibroblasts; i.e. the CG-1 NPC-derived cell line.[62] There have been many limitations in culturing these cell lines. The following are some problems encountered when trying to establish an EBV-positive NPC cell lines. Firstly, there are not many true EBV-positive NPC cell lines available that can be used as a standard for the development of other NPC cell lines with the only exception of C-666.[63] However, its status as a true NPC cell line remains questionable. Secondly, the cultures lose their EBV- positively after long periods of *in vitro* culture. Transplantation into nude mice of NPC material has not been very efficacious and most of the primary transplanted tumours are no longer available for further investigation.[64] Moreover, it is best if a tumour is obtained directly from primary tissue rather than from another source such as from xenograft or transformed EBV+ cell lines. This provides a more accurate reflection of the cytogenetic details of the primary tumour and thus is a better tool for study of NPC tumourigenicity.

Conclusion

Here we have presented the importance of using authentic EBV+ human NPC cell lines for research in NPC. Specifically, continuous cell lines are important tools for the study of the genetic mechanisms and environmental factors responsible for NPC, both in terms of tumourigenicity as well as its metastatic potential. Although, there were many NPC cell lines that have been established using numerous culture methods, most of them are EBV-negative after a long-term culture. Carcinomas with similar features to UNPC have been described at other sites including the thymus, tonsils, lungs, stomach, skin or cervix, and are often referred to as undifferentiated carcinoma of nasopharyngeal type (UCNT).[86] Some of these carcinomas showed less strong EBV-positively, such as those derived from thymic epithelia tumours from Chinese but not western patients.[87]

The precise mechanism by which EBV transforms cells is becoming clearer as more research is conducted. The viral gene expression in EBV-associated tumours is limited to a few latent genes of which the most prevalent is LMP1. It is reported to act as a constitutively activated receptor that causes aberrant cellular signalling to at least 4 major pathways. The role for virus in the pathogenesis of NPC is still largely circumstantial and controversial.[88] For example, the squamous cell NPCs and non-keratinising NPCs vary in their degree of EBV association. Squamous cell NPCs may in fact be EBV-negative and other factors such as smoking or HPV infection may be more relevant in the

pathogenesis of these cell types. On the other hand, non-keratinising NPCs are almost always EBV-positive, suggesting that the virus is a rate-limiting step for these tumours. It will be important to define the exact timing of EBV infection of nasopharyngeal epithelial cells and to identify the factors that precede and probably pave the way for EBV infection. Clearly, further studies are required to support these findings. The complexity of these virus and host relationships suggests the existence of potential targets for gene therapy for EBV-related cancers.

The geographically constrained distribution of EBV associated NPC in Southeast Asian populations suggests that both viral and host genetics may influence disease risk. The analysis of LMP1 with reference to the geographical distribution demonstrate that LMP1 sequences show a distinct geographic structure, indicating that the southeast Asian isolates have evolved as a lineage distinct from those of Papua New Guinea, African, and Australian isolates.[89] Due to the fact that NPC occurs more frequently in Asia than compared to the Western regions, there is increased motivation in establishing NPC cell lines from each region.

Acknowledgements

This work was supported by research grants from the National Medical Research Council (NMRC), Singapore (grant # NMRC/0676/2002) and SGH (grant # SGHRF#58/01). We would like to acknowledge Ms Wan Teng Sing for all the hard work she has done in the laboratory to try and derive NPC cell lines and for her help with this manuscript.

REFERENCES

1. Ballinger JR, Li JH. Sestamibi accumulation in human nasopharyngeal carcinoma cell lines in vitro. Anticancer Res 2000;20:677-9.
2. Cohen JI. Epstein-Barr virus infection. N Engl J Med 2000;17:481-92.
3. Huang ES, Gutsch D, Tzung KW, Lin CT. Detection of low level of human papilloma virus type 16 DNA sequences in cancer cell lines derived from two well-differentiated nasopharyngeal cancers. J Med Virol 1993;40:244-50.
4. Hsu JL, Glaser SL. Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications. Crit Rev Oncol Hematol 2000;34:27-53.
5. Yu MC. Diet and nasopharyngeal carcinoma. FEMS Microbiol Immunol 1990;2:235-42.
6. Yu MC. Nasopharyngeal carcinoma: epidemiology and dietary factors. IARC Sci Publ 1991;39-47.
7. Poirier S, Ohshima H, de The G, Hubert A, Bourgade MC, Bartsch H. Volatile nitrosamine levels in common foods from Tunisia, south China and Greenland, high-risk areas for nasopharyngeal carcinoma (NPC). Int J Cancer 1987;39:293-6.
8. Wu YT, Luo HL, Johnson DR. Effect of nickel sulfate on cellular proliferation and Epstein-Barr virus antigen expression in lymphoblastoid cell lines. Cancer Lett 1986;32:171-9.
9. Armstrong RW, Imrey PB, Lye MS, Armstrong MJ, Yu MC, Sani S. Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. Int J Epidemiol 2000;29:991-8.
10. Stevens CR, Rassekh C. Nasopharyngeal Carcinoma. Dept.of Otolaryngology, UTMB, Galveston, TX. 21-1-1998. Ref Type: Internet Communication
11. Pathmanathan R, Prasad U, Sadler R, Flynn K, Raab-Traub N. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med 1995;333: 693-8.
12. Chia KS, Seow A, Lee HP, Shanmugaratnam. Cancer Incidence in Singapore 1993-1997. Singapore: Singapore Cancer Registry, 2000;5: 72-3.
13. Niedobitek G, Hansmann ML, Herbst H, Young LS, Dienemann D, Hartmann CA, et al. Epstein-Barr virus and carcinomas: undifferentiated carcinomas but not squamous cell carcinomas of the nasopharynx are regularly associated with the virus. J Pathol 1991;165:17-24.
14. Weber AL, al Arayedh S, Rashid A. Nasopharynx: clinical, pathologic, and radiologic assessment. Neuroimaging Clin N Am 2003;13:465-83.
15. Epstein Ma, Achong Bg, Barr Ym. Virus particles in cultured lymphoblasts from burkitt's lymphoma. Lancet 1964;15:702-3.
16. Baumforth KR, Young LS, Flavell KJ, Constandinou C, Murray PG. The Epstein-Barr virus and its association with human cancers. Mol Pathol 1999;52:307-22.
17. Niedobitek G, Fahraeus R, Herbst H, Latza U, Ferszt A, Klein G, et al. The Epstein-Barr virus encoded membrane protein (LMP) induces phenotypic changes in epithelial cells. Virchows Arch B Cell Pathol Incl Mol Pathol 1992;62:55-9.
18. Young LS, Dawson CW, Clark D, Rupani H, Busson P, Tursz T, et al. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. J Gen Virol 1988;69:1051-65.
19. Chang YS, Tyan YS, Liu ST, Tsai MS, Pao CC. Detection of Epstein-Barr virus DNA sequences in nasopharyngeal carcinoma cells by enzymatic DNA amplification. J Clin Microbiol 1990;28:2398-402.
20. Old LJ, Boyse EA, Oettgen HF, Harven ED, Geering G, Williamson B, et al. Precipitating antibody in human serum to an antigen present in cultured burkitt's lymphoma cells. Proc Natl Acad Sci U S A 1966; 56:1699-704.
21. Shao YM, Poirier S, Ohshima H, Malaveille C, Zeng Y, de The G, et al. Epstein-Barr virus activation in Raji cells by extracts of preserved food from high risk areas for nasopharyngeal carcinoma. Carcinogenesis 1988;9:1455-7.
22. Raab-Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. Cell 1986;47:883-9.
23. Klein G. The relationship of the virus to nasopharyngeal carcinoma. In: Epstein Ma, Achong Bg, editors. The Epstein-Barr Virus. Berlin: Springer-Verlag; 1979:339-50.
24. Weiss LM, Movahed LA, Butler AE, Swanson SA, Frierson HF, Jr., Cooper PH, et al. Analysis of lymphoepithelioma and lymphoepithelioma-like carcinomas for Epstein-Barr viral genomes by in situ hybridization. Am J Surg Pathol 1989;13:625-31.
25. Billaud M, Busson P, Huang D, Mueller-Lantzch N, Rousselet G, Pavlish O, et al. Epstein-Barr virus (EBV)-containing nasopharyngeal carcinoma cells express the B-cell activation antigen blast2/CD23 and low levels of the EBV receptor CR2. J Virol 1989;63:4121-8.
26. de The G, Ablashi DV, Favre MC, Mourali N, Ellouz R. Presence of Epstein-Barr virus nuclear antigen in nasopharyngeal carcinoma biopsies and their derived cultures. Bibl Haematol 1975;40:101-3.
27. Low WK, Leong JL, Goh YH, Fong KW. Diagnostic value of Epstein-Barr viral serology in nasopharyngeal carcinoma. Otolaryngol Head Neck Surg 2000;123:505-7.
28. Brooks L, Yao QY, Rickinson AB, Young LS. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1, and LMP2 transcripts. J Virol 1992;66:2689-97.
29. Gilligan KJ, Rajadurai P, Lin JC, Busson P, Abdel-Hamid M, Prasad U, et al. Expression of the Epstein-Barr virus BamHI A fragment in

nasopharyngeal carcinoma: evidence for a viral protein expressed in vivo. J Virol 1991;65:6252-9.
30. Brooks LA, Lear AL, Young LS, Rickinson AB. Transcripts from the Epstein-Barr virus BamHI A fragment are detectable in all three forms of virus latency. J Virol 1993;67:3182-90.
31. Deacon EM, Pallesen G, Niedobitek G, Crocker J, Brooks L, Rickinson AB, et al. Epstein-Barr virus and Hodgkin's disease: transcriptional analysis of virus latency in the malignant cells. J Exp Med 1993;177: 339-49.
32. Cochet C, Martel-Renoir D, Grunewald V, Bosq J, Cochet G, Schwaab G, et al. Expression of the Epstein-Barr virus immediate early gene, BZLF1, in nasopharyngeal carcinoma tumor cells. Virology 1993; 197:358-65.
33. Mazeron MC. [Value of anti-Epstein-Barr antibody detection in the diagnosis and management of undifferentiated carcinoma of the nasopharynx]. Bull Cancer Radiother 1996;83:3-7.
34. Zheng X, Yan L, Nilsson B, Eklund G, Drettner B. Epstein-Barr virus infection, salted fish and nasopharyngeal carcinoma. A case-control study in southern China. Acta Oncol 1994;33:867-72.
35. Wu HC, Lin YJ, Lee JJ, Liu YJ, Liang ST, Peng Y, et al. Functional analysis of EBV in nasopharyngeal carcinoma cells. Lab Invest 2003;83:797-812.
36. Ardila-Osorio H, Clausse B, Mishal Z, Wiels J, Tursz T, Busson P. Evidence of LMP1-TRAF3 interactions in glycosphingolipid-rich complexes of lymphoblastoid and nasopharyngeal carcinoma cells. Int J Cancer 1999;81:645-9.
37. Eliopoulos AG, Dawson CW, Mosialos G, Floettmann JE, Rowe M, Armitage RJ, et al. CD40-induced growth inhibition in epithelial cells is mimicked by Epstein-Barr Virus-encoded LMP1: involvement of TRAF3 as a common mediator. Oncogene 1996;13:2243-54.
38. Floettmann JE, Eliopoulos AG, Jones M, Young LS, Rowe M. Epstein-Barr virus latent membrane protein-1 (LMP1) signalling is distinct from CD40 and involves physical cooperation of its two C-terminus functional regions. Oncogene 1998;17:2383-92.
39. Dawson CW, Rickinson AB, Young LS. Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. Nature 1990;344:777-80.
40. Eliopoulos AG, Stack M, Dawson CW, Kaye KM, Hodgkin L, Sihota S, et al. Epstein-Barr virus-encoded LMP1 and CD40 mediate IL-6 production in epithelial cells via an NF-kappaB pathway involving TNF receptor-associated factors. Oncogene 1997;14:2899-916.
41. Hintzen RQ, Lens SM, Koopman G, Pals ST, Spits H, van Lier RA. CD70 represents the human ligand for CD27. Int Immunol 1994;6:477-80.
42. Takeshita H, Yoshizaki T, Miller WE, Sato H, Furukawa M, Pagano JS, et al. Matrix metalloproteinase 9 expression is induced by Epstein-Barr virus latent membrane protein 1 C-terminal activation regions 1 and 2. J Virol 1999;73:5548-55.
43. Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, Arnold W, et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. J Clin Invest 1997;100:2961-9.
44. He Z, Xin B, Yang X, Chan C, Cao L. Nuclear factor-kappaB activation is involved in LMP1-mediated transformation and tumorigenesis of rat-1 fibroblasts. Cancer Res 2000;60:1845-8.
45. Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: Fields BN, Howley PM, Griffin DE, et al, editors. Field's Virology. 4th edition. Philadelphia: Lippincott,Williams & Wilkins, 2001:2511-73.
46. Tsao SW, Tramoutanis G, Dawson CW, Lo AK, Huang DP. The significance of LMP1 expression in nasopharyngeal carcinoma. Semin Cancer Biol 2002;12:473-87.
47. Morrison JA, Gulley ML, Pathmanathan R, Raab-Traub N. Differential signaling pathways are activated in the Epstein-Barr virus-associated malignancies nasopharyngeal carcinoma and Hodgkin lymphoma. Cancer Res 2004;64:5251-60.
48. Rowe M, Rowe DT, Gregory CD, Young LS, Farrell PJ, Rupani H, et al. Differences in B cell growth phenotype reflect novel patterns of Epstein-Barr virus latent gene expression in Burkitt's lymphoma cells. EMBO J 1987;6:2743-51.
49. Young LS, Murray PG. Epstein-Barr virus and oncogenesis: from latent genes to tumours. Oncogene 2003;22:5108-21.
50. Yates J, Warren N, Reisman D, Sugden B. A cis-acting element from the Epstein-Barr viral genome that permits stable replication of recombinant plasmids in latently infected cells. Proc Natl Acad Sci U S A 1984;81: 3806-10.
51. Johannsen E, Koh E, Mosialos G, Tong X, Kieff E, Grossman SR. Epstein-Barr virus nuclear protein 2 transactivation of the latent membrane protein 1 promoter is mediated by J kappa and PU.1. J Virol 1995;69: 253-62.
52. Wang D, Liebowitz D, Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell 1985;43:831-40.
53. Uchida J, Yasui T, Takaoka-Shichijo Y, Muraoka M, Kulwichit W, Raab-Traub N, et al. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. Science 1999;286:300-3.
54. Henderson S, Rowe M, Gregory C, Croom-Carter D, Wang F, Longnecker R, et al. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991;65:1107-15.
55. Eliopoulos AG, Gallagher NJ, Blake SM, Dawson CW, Young LS. Activation of the p38 mitogen-activated protein kinase pathway by Epstein-Barr virus-encoded latent membrane protein 1 coregulates interleukin-6 and interleukin-8 production. J Biol Chem 1999;274: 16085-96.
56. Gires O, Zimber-Strobl U, Gonnella R, Ueffing M, Marschall G, Zeidler R, et al. Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule. EMBO J 1997;16:6131-40.
57. Eliopoulos AG, Davies C, Blake SS, Murray P, Najafipour S, Tsichlis PN, et al. The oncogenic protein kinase Tpl-2/Cot contributes to Epstein-Barr virus-encoded latent infection membrane protein 1-induced NF-kappaB signaling downstream of TRAF2. J Virol 2002;76:4567-79.
58. Sylla BS, Hung SC, Davidson DM, Hatzivassiliou E, Malinin NL, Wallach D, et al. Epstein-Barr virus-transforming protein latent infection membrane protein 1 activates transcription factor NF-kappaB through a pathway that includes the NF-kappaB-inducing kinase and the IkappaB kinases IKKalpha and IKKbeta. Proc Natl Acad Sci U S A 1998;95: 10106-11.
59. Frade R. Structure and functions of gp 140, the C3d/EBV receptor (CR2) of human B lymphocytes. Mol Immunol 1986;23:1249-53.
60. Glaser R, Zhang HY, Yao KT, Zhu HC, Wang FX, Li GY, et al. Two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus that were derived from nasopharyngeal carcinomas. Proc Natl Acad Sci U S A 1989;86:9524-8.
61. Yao KT, Zhang HY, Zhu HC, Wang FX, Li GY, Wen DS, et al. Establishment and characterization of two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus and derived from nasopharyngeal carcinomas. Int J Cancer 1990;45:83-9.
62. Chang YS, Lin SY, Lee PF, Durff T, Chung HC, Tsai MS. Establishment and characterization of a tumor cell line from human nasopharyngeal carcinoma tissue. Cancer Res 1989;49:6752-7.
63. Hui AB, Cheung ST, Fong Y, Lo KW, Huang DP. Characterization of a new EBV-associated nasopharyngeal carcinoma cell line. Cancer Genet Cytogenet 1998;101:83-8.
64. Busson P, Ganem G, Flores P, Mugneret F, Clausse B, Caillou B, et al. Establishment and characterization of three transplantable EBV-containing nasopharyngeal carcinomas. Int J Cancer 1988;42:599-606.
65. Chen W, Lee Y, Wang H, Yu GG, Jiao W, Zhou W, et al. Suppression of human nasopharyngeal carcinoma cell growth in nude mice by the wild-type p53 gene. J Cancer Res Clin Oncol 1992;119:46-8.
66. Chen Y, Guo H, Wang H. [Effect of EBV latent membrane protein 1 gene isolated from human nasopharyngeal carcinoma cell line SUNE on the growth of immortalized epithelial cells]. Zhonghua Zhong Liu Za Zhi 1998;20:330-2.

67. Cheung ST, Huang DP, Hui AB, Lo KW, Ko CW, Tsang YS, et al. Nasopharyngeal carcinoma cell line (C666-1) consistently harbouring Epstein-Barr virus. Int J Cancer 1999;83:121-6.
68. Hu LF, Zabarovsky ER, Chen F, Cao SL, Ernberg I, Klein G, et al. Isolation and sequencing of the Epstein-Barr virus BNLF-1 gene (LMP1) from a Chinese nasopharyngeal carcinoma. J Gen Virol 1991;72: 2399-409.
69. Huang DP, Ho JH, Chan WK, Lau WH, Lui M. Cytogenetics of undifferentiated nasopharyngeal carcinoma xenografts from southern Chinese. Int J Cancer 1989;43:936-9.
70. Lin CT, Chan WY, Chen W, Huang HM, Wu HC, Hsu MM, et al. Characterization of seven newly established nasopharyngeal carcinoma cell lines. Lab Invest 1993;68:716-27.
71. Lin CT, Dee AN, Chen W, Chan WY. Association of Epstein-Barr virus, human papilloma virus, and cytomegalovirus with nine nasopharyngeal carcinoma cell lines. Lab Invest 1994;71:731-6.
72. Sizhong Z, Xiukung G, Yi Z. Cytogenetic studies on an epithelial cell line derived from poorly differentiated nasopharyngeal carcinoma. Int J Cancer 1983;31:587-90.
73. Wang X, Masters JR, Wong YC, Lo AK, Tsao SW. Mechanism of differential sensitivity to cisplatin in nasopharyngeal carcinoma cells. Anticancer Res 2001;21:403-8.
74. Yao KT. [Establishment of a novel cell line derived from nasopharyngeal carcinoma]. Zhonghua Zhong Liu Za Zhi 1990;12:34-6.
75. Zhang HY, Yao K, Zhu HC, Glaser R. Expression of the Epstein-Barr virus genome in a nasopharyngeal carcinoma epithelial tumor cell line. Int J Cancer 1990;46:944-9.
76. Zhao ML, Luo CM, Long F, Chen J, Tang LY. [Growth capability of epithelial cell line of human poorly differentiated nasopharyngeal carcinoma and its response to Chinese medicinal herbs and marine drugs]. Zhonghua Zhong Liu Za Zhi 1988;10:98-101.
77. Lin CT, Wong CI, Chan WY, Tzung KW, Ho JK, Hsu MM, et al. Establishment and characterization of two nasopharyngeal carcinoma cell lines. Lab Invest 1990;62:713-24.
78. Tsukuda M, Kubota A, Kaneko M, Yoshida T, Sawaki S. [The characteristics of nasopharyngeal carcinoma cell strain against chemotherapeutic agents and cytokines]. Nippon Jibiinkoka Gakkai Kaiho 1991;94:28-33.
79. Teng ZP, Ooka T, Huang DP, Zeng Y. Detection of Epstein-Barr Virus DNA in well and poorly differentiated nasopharyngeal carcinoma cell lines. Virus Genes 1996;13:53-60.
80. Gao J, Young G, Xue KX, Li BG, Sun YL. Characteristics of invasiveness of human nasopharyngeal carcinoma cells in organ culture, as observed by scanning electron microscopy. Pathol Res Pract 1982;174:325-41.
81. Huang DP, Ho JH, Poon YF, Chew EC, Saw D, Lui M, et al. Establishment of a cell line (NPC/HK1) from a differentiated squamous carcinoma of the nasopharynx. Int J Cancer 1980;26:127-32.
82. Unknown, no authors listed. Establishment of an epitheloid cell line and a fusiform cell line from a patient with nasopharyngeal carcinoma. Sci Sin 1978;21:127-34.
83. Cao SL, Liu TF, Wang ZH, Huang KM, Zhou J, Li DL, et al. [Flow cytometer (FCM) in biological characteristics of nasopharyngeal carcinoma xenografts in nude mice]. Zhonghua Zhong Liu Za Zhi 1987;9:6-9.
84. Fahraeus R, Fu HL, Ernberg I, Finke J, Rowe M, Klein G, et al. Expression of Epstein-Barr virus-encoded proteins in nasopharyngeal carcinoma. Int J Cancer 1988;42:329-38.
85. Furukawa M, Kamide M, Miwa T, Sakumoto M, Umeda R. Epstein-Barr virus (EBV) infection by cocultivation of fibroblast-dominant cell line and EBV-transformed lymphocytes. ORL J Otorhinolaryngol Relat Spec 1989;51:69-76.
86. Spano JP, Busson P, Atlan D, Bourhis J, Pignon JP, Esteban C, et al. Nasopharyngeal carcinomas: an update. Eur J Cancer 2003;39: 2121-35.
87. Fujii T, Kawai T, Saito K, Fukushima K, Hasegawa T, Tokunaga M, et al. EBER-1 expression in thymic carcinoma. Acta Pathol Jpn 1993;43: 107-10.
88. Unknown- no authors listed. Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Epstein-Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8. Lyon, France, 17-24 June 1997. IARC Monogr Eval Carcinog Risks Hum 1997;70:1-492.
89. Burrows JM, Bromham L, Woolfit M, Piganeau G, Tellam J, Connolly G, et al. Selection pressure-driven evolution of the Epstein-Barr virus-encoded oncogene LMP1 in virus isolates from Southeast Asia. J Virol 2004;78:7131-7.
90. Huang D, Lau WH, Luo CM, Saw D, Lau WH. Establishment and characterization of undifferentiated nasopharyngeal carcinoma xenografts from southern chinese. J Exp Clin Cancer Res 1988;7:48-52.
91. Liao SK, Perng YP, Shen YC, Chung PJ, Chang YS, Wang CH. Chromosomal abnormalities of a new nasopharyngeal carcinoma cell line (NPC-BM1) derived from a bone marrow metastatic lesion. Cancer Genet Cytogenet 1998;103:52-8.