An Update on Epstein-Barr Virus and Nasopharyngeal Carcinogenesis

Bayardo Perez-Ordóñez

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Epstein-Barr Virus

Epstein-Barr virus (EBV) is a double stranded DNA γ -herpesvirus with widespread distribution in all human populations. EBV is associated with a variety of diseases including infectious mononucleosis, hairy leukoplakia, inflammatory pseudotumors, nasopharyngeal carcinoma (NPC), Burkitt's lymphoma, Hodgkin lymphoma, post-transplant lymphoproliferative disorders, HIV-associated B-cell lymphomas, some T-cell lymphomas particularly extranodal NK/T cell lymphomas of the nasal-type, and a subset of gastric and breast carcinomas. EBV preferentially infects B-lymphocytes through the binding of the major envelop glycoprotein gp350 to the CD21 receptor on the surface of B-cells and through the binding of a second glycoprotein, gp42, to human leukocyte antigen (HLA) class II molecules as a co-receptor [1]. EBV has the capacity to transform resting B-cells into permanent latently infected lymphoblastoid cell lines.

Epstein-Barr virus-transformed lymphoblastoid cell lines express a set of viral gene products referred to as latent proteins which include six EBV nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C, -LP) and three latent membrane proteins (LMPs 1, 2A, and 2B). Transformed lymphoblastoid cells also show abundant expression of small, non-polyadenylated, non-coding RNAs (EBER1 and EBER2) which are expressed in all forms of latent EBV infection.

Transcripts from the *Bam*HIA viral genome known as BART-transcripts are also detected in lymphoblastoid cells [2]. EBNA2, EBNA3C and LMP1 are key in the transformation of EBV-infected cells [3, 4]. LMP1 is the main transforming protein of EBV and functions as a classic oncogene in fibroblast transformation assay [5]. LMP1 functions as an activated member of the tumor receptor (TNFR) superfamily, and activates several signaling pathways [6, 7].

Epstein-Barr Virus and Nasopharyngeal Carcinoma

Epstein-Barr virus (EBV) latent infection of normal epithelial cells has not been detected in normal cells. The mechanisms of EBV latent infection of epithelial cells are not well understood, but a serious consequence of epithelial latent EBV infection is malignant transformation resulting in the development of NPC, a subset of gastric and breast carcinomas and certain salivary gland carcinomas. Studies of normal nasopharyngeal tissue and premalignant samples in patients at high risk for the development of NPC indicate that genetic events—particularly *RASSF1A* and *p16* inactivation—occur early in the pathogenesis of NPC, and that these events might predispose the abnormal epithelium to subsequent EBV infection originating from adjacent lymphoid tissues and circulating B-cells [8]. EBV latent-gene expression in NPC is predominantly restricted to the EBNA1 nuclear antigen, the latent membrane proteins LMP2A and LMP2B, *Bam*HIA transcripts, and the oncogenic LMP1 protein and is consistent with a type II latency [8, 9]. Southern-blot hybridization from NPC tissues demonstrates monoclonality of the viral genome indicating that the EBV infection takes place before the clonal expansion of the malignant cells [10, 11].

B. Perez-Ordóñez (✉)
Department of Pathology, University Health Network,
200 Elizabeth Street, Toronto, Ontario, Canada M5G 2C4
e-mail: bayardo.perez-ordonez@uhn.on.ca

B. Perez-Ordóñez
Department of Pathobiology and Laboratory Medicine,
University of Toronto, Toronto, Ontario, Canada

Epstein-Barr Virus Detection

Tests for the detection of EBV and their main applications are summarized in Table 1. Serological studies identify increased EBV-specific antibody titers in individuals living in high-incidence areas and in patients affected by NPC. The most common serologic studies employed in clinical practice include IgA antibodies against viral capsid antigen (VCA) and IgG/IgA antibodies against early antigens (EA) [12]. Antibodies to the EBV capsid antigen have proved useful in monitoring the effectiveness of therapy (Fig. 1) [13, 14]. More recent studies using real-time quantitative PCR to measure circulating tumor derived EBV DNA in the blood of patients with NPC have shown that the levels of pre-treatment EBV DNA are strongly associated with overall survival, and that post-treatment EBV DNA predict progression free and overall survival [13, 15].

The simplest and more reliable method for detection of EBV in tissues is in-situ hybridization for EBV encoded early RNAs (EBERs) [12, 16]. In-situ hybridization allows identification of the infected cells in tissue sections. In NPC practically all the tumor cells show nuclear staining. EBV latent membrane protein-1 (LMP-1) immunohistochemistry is less reliable and often is weak and patchy with expression being detected in less than 40% of cases. PCR detection of EBV is unreliable since even the presence of a few non-neoplastic EBV-infected lymphocytes will yield a positive result.

Nasopharyngeal Carcinoma Precursor Lesions

Although NPC in-situ can be identified in approximately less than 10% of conventional invasive NPC, pure

Fig. 1 Patient with T3N1M0 nasopharyngeal carcinoma with 8,667 EBV-DNA copies/ml before combined chemoradiation. The EBV-DNA levels dropped to 238 copies/ml after completion of therapy but increased to 17,333 copies/ml after the development of pulmonary metastases. (Courtesy of Dr. Suzanne Kamel-Reid)

nasopharyngeal carcinoma in-situ is exceedingly rare [10, 17, 18]. As invasive NPC, nasopharyngeal carcinoma in-situ is positive for EBV (EBER) (Fig. 2), a finding that supports the concept that EBV infection in an early event in NPC carcinogenesis [10, 17, 18]. The absence of EBV-infected epithelial cells in normal nasopharyngeal mucosa from individuals at high risk of developing NPC argues against a pre-existing normal reservoir infected cells from which virus-positive carcinoma arise; however, deletions in chromosome regions 3p and 9p have been identified in low-grade dysplastic lesions and normal nasopharyngeal mucosa of individuals at high risk of developing NPC indicating that these genetic events occur early in the pathogenesis of NPC and that they might cause predisposition to subsequent EBV infection [19, 20].

Table 1 Epstein-Barr virus detection in the laboratory (Gulley 2001) [12]

Test	Main applications
In-situ hybridization (EBER)	Identify EBER transcripts or EBV DNA in specific cell types in microscopic sections
EBV DNA amplification	Assess clonality of lesions Distinguish latent from replicative infection based on the episomal (circular) versus linear EBV genome
Serology (VCA, EBNA, EA, heterophile antibodies)	Measures antibody response to viral proteins in serum samples Distinguishes acute from remote infection Monitor disease status
EBV viral load	Quantitate EBV DNA in blood or fluids to monitor disease status
Immunohistochemistry (LMP1, EBNA1, EBNA2, LMP2A, BZLF1)	Identify EBV protein expression in specific cell types in microscopic sections Distinguished latent from replicative infection based on expression profiles
Culture of EBV or EBV-infected B-cells	Detect and quantify infectious virions or latently-infected B-cells Impractical for routine clinical use
Electron microscopy	Identify whole virions representing replicative viral infection Impractical for routine clinical use

Fig. 2 Nonkeratinizing nasopharyngeal carcinoma in-situ with strong EBERs expression by ISH (inset)

Pathologic Classification of Nasopharyngeal Carcinoma

The morphologic features of nasopharyngeal carcinoma have been well described and summarized in the 2005

WHO Classification of Head and Neck Tumors (Table 2) (Fig. 3) [21]. Subclassification of nonkeratinizing carcinoma into undifferentiated and differentiated subtypes is optional since their distinction is of no clinical or prognostic significance and features of both types can be seen in the same biopsy material or in sequential biopsies from the same patient. Nonkeratinizing and keratinizing squamous carcinomas are almost invariably associated with EBV in all geographic areas [22], whereas only a small proportion of keratinizing squamous cell carcinoma are positive for EBV in areas with low incidence of NPC. EBV expression in keratinizing squamous cell carcinomas tend to be low and limited to cells with a basaloid appearance with terminally differentiated squamous cells being negative. There has been an overall decline in the incidence of NPC in Hong Kong, but the decline appears to be limited to a decrease in keratinizing squamous cell carcinoma attributable to a decrease in smoking and other environmental factors [23].

Table 2 2005 WHO Classification of nasopharyngeal carcinoma and incidence of histologic types [21]

WHO histologic types	High incidence population	Intermediate incidence population	Low incidence population
Keratinizing squamous cell carcinoma	1–17%	8%	13–25%
Nonkeratinizing carcinoma	83–99%	92%	75%
-Undifferentiated			
-Differentiated			
Basaloid squamous cell carcinoma	<0.2%	–	–

– Unknown

Fig. 3 Surgical pathology of nasopharyngeal carcinoma. Nonkeratinizing carcinoma, differentiated-type composed of intermediate size cells with well defined eosinophilic cytoplasm and oval nuclei lacking prominent nucleoli. This case shows formation of a whorl (A). Nonkeratinizing carcinoma, undifferentiated-type is composed of

large cells with amphophilic cytoplasm showing vesicular chromatin and prominent nucleoli (B). Keratinizing squamous carcinoma accompanied by carcinoma in-situ (C). Basaloid squamous cell carcinoma showing composed of small cells with peripheral palisading and formation of eosinophilic cylinders of basal lamina material (D)

Molecular Pathology of Nasopharyngeal Carcinoma

Genetic Risk

There is an association between certain HLA phenotypes and increased risk of developing NPC. These haplotypes include HLA A2-B46, HLA B17, HLA A2-B38, and HLA A2-B16 [24, 25]. The genetic bases of familial NPC are not well understood, but susceptibility loci have been identified in chromosome regions 3p21 and 4p15 [26, 27].

GSTM1 and CYP2E1 Polymorphisms

Glutathione *S*-transferase M1 (GSTM1) detoxifies benzo[a]pyrene and the cytochrome P450 2E1 is responsible for the metabolic activation of carcinogenic nitrosamines. Although the literature remains controversial, it has been reported that alterations of GSTM1 and CYP2E1 are associated with a moderate increased risk of NPC [28, 29].

Molecular Genetic Alterations

Molecular alterations in NPC are complex (Fig. 4) (Table 3). Inactivation of the *RASSF1A* and *p16* tumor suppressor genes on 3p21 and 9p21 by homozygous deletions and promoter methylation are the most common alterations described in NPC [30–32]. 3p and 9p abnormalities have been identified in low-grade dysplastic lesions and normal nasopharyngeal mucosa of individuals at high risk indicating that these genetic changes are early events in the pathogenesis of NPC [10, 17, 20, 33]. Other genes frequently inactivated by promoter methylation in NPC include *TSCL1* at 11q23 and *EDNRB* at 13q22,

Fig. 4 Proposed model of nasopharyngeal carcinogenesis with progressive accumulation of genetic and epigenetic abnormalities and corresponding histopathologic and clinical features

Table 3 Common molecular abnormalities in NPC

<i>RASSF1A</i> promoter methylation
<i>p16</i> homozygous deletions and methylation
<i>EDNRB</i> promoter methylation
<i>TSCL1</i> promoter methylation
E-cadherin hypermethylation
DAPK methylation
Telomerase dysregulation

E-cadherin, and death-associated protein kinase (DAPK) [34–36]. Gene expression profiling has shown dysregulation of the PI3K/Akt, WNT/ β -catenin, *TGF- β* , and *MAPK* signaling pathways in NPC with upregulation of NF- κ B2, survivin, Bcl-2 upregulation, nuclear accumulation of β -catenin, and dysregulation of integrins [37].

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