Biomarkers in Nasopharyngeal Carcinoma and Ionizing Radiation

39

Thian-Sze Wong, Wei Gao, and Jimmy Yu-Wai Chan

Contents

T.-S. Wong (✉) • W. Gao
Department of Surgery, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong
e-mail: thiansze@gmail.com; thiansze@graduate.hku.hk

J.Y.-W. Chan
Department of Surgery, Queen Mary Hospital, The University of Hong Kong, Hong Kong, Hong Kong
e-mail: chanjyw@gmail.com

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Abstract

Cancer of the nasopharynx is an epithelial cancer developed in the retro-nasal area in the nasal cavity. Nasopharyngeal carcinoma (NPC) is prevalent in the endemic regions including Southern China and Southeast Asia. The primary tumor of NPC is small and usually causes no symptoms. Patients often present with bilateral glands enlargement and local/regional lymph node metastasis leading to poor prognosis and local control rate. Therefore, there is a desperate need to develop early diagnosis method to improve the treatment outcome. Detection of EBV-derived biomarkers in tissues and EBV DNA in the peripheral blood of NPC patients opens up the possibility to monitor the disease using molecular markers with high sensitivity. Among the biomarkers in tissue, in situ hybridization for EBER remains to be the most efficient and reliable way because EBER is the most abundantly expressed viral transcript. In addition to diagnosis value, EBER, EBNA1, and LMP1 levels in tissue after radiation therapy could serve as biomarkers to evaluate response to radiotherapy. In light of the detection of EBV DNA in plasma, serum, nasal brush, and saliva, EBV DNA is particularly useful for periodic monitoring of NPC patients. A systematic review has revealed that circulating EBV DNA could be applied as noninvasive diagnostic biomarker for NPC. Moreover, the close association of circulating EBV DNA with the clinical outcomes of treatment provided new tool to predict the treatment outcome. Nevertheless, circulating EBV DNA was not detectable in all the undifferentiated NPC patients, and EBV latent infection was not associated with WHO-1 NPC, which limits the utility of EBV DNA. Further studies are warranted to identify complementary biomarkers originating from the human cancer cells to overcome the limitation of EBV-based biomarkers in NPC screening and monitoring treatment outcome.

List of Abbreviations

CT	Computerized Tomography
EA	Early Antigen
EBER	Epstein–Barr-Encoded RNA
EBNA	Epstein–Barr Nuclear Antigen
EBV	Epstein–Barr Virus
IARC	International Agency for Research on Cancer
IM	Infectious Mononucleosis
LMP	Latent Membrane Protein
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
NPC	Nasopharyngeal Carcinoma
VCA	Viral Capsid Antigen
WHO	World Health Organization

Key Facts

Key Facts of NPC

High incidence occurs in Southeastern Asia including Southeastern China, Thailand, Malaysia, and Indonesia.
It is predominant in male patients.
Most symptoms of NPC are vague and nonspecific at the early stages.
By the time of diagnosis, the patients are usually at the advanced stages.
Radiotherapy is recommended for the mainstay treatment regime as the cancer cells demonstrated high sensitivity to radiation.
Cisplatin-based concurrent chemoradiotherapy is recommended for advanced disease.
NPC in the endemic areas is characterized by its consistent association with the EBV.

Key Facts of EBV

It is a double-stranded DNA virus and was first identified in Burkitt's lymphoma cells in 1964.
It belongs to the herpes family and is one of the most common viruses in human.
EBV is capable of selecting two different lifestyles including latent replication and lytic replication.
Since it exerts causal roles in human malignancies, it is classified as group I carcinogen.
The closed association between EBV and NPC was observed, and EBV could serve as a biomarker for diagnosis and monitoring of NPC.
In addition to NPC, EBV was associated with a wide variety of human malignancies such as Hodgkin's disease, non-Hodgkin's lymphoma, and gastric adenocarcinoma.

Definition of Words and Terms

NPC NPC is an epithelial cancer occurring in the retro-nasal area in the nasal cavity, which is predominant in male patients and prevalent in Southeastern Asia.

EBV EBV is a double-stranded DNA virus and belongs to the herpes family, which exerts causal roles in human malignancies such as NPC, Hodgkin's disease, non-Hodgkin's lymphoma, and gastric adenocarcinoma.

Lifestyles of EBV EBV is capable of selecting two different lifestyles including latent replication or lytic replication, and induction of lytic form of EBV infection results in destruction of malignancies with latently infected EBV.

Latent phase of EBV The EBV genomic DNA presents as a closed circular plasmid and is replicated only once during S phase. Viral DNA maintenance requires oriP cis element, EBNA1 protein, and chromosomal initiation factors.

Lytic phase of EBV Lytic phase is a viral productive cycle in which multiple rounds of replication occur. Two key EBV immediate early lytic genes, BZLF1 and BRLF1, activate lytic replication.

Epstein–Barr-encoded RNA They are noncoding RNA transcripts which exhibited the highest abundance among viral transcripts; thus, they represent the most efficient and reliable method to detect EBV in biopsy samples.

Epstein–Barr nuclear antigen 1 It is a DNA-binding protein and acts as transcription activator to enhance EBV gene expression. It is essential for maintaining EBV genome in host cells by protecting the virus from being recognized by the immune system.

Latent membrane protein 1 It is an integral membrane protein associated with advanced and metastatic disease. It contributes to aggressive phenotype in NPC and is a promising therapeutic target for NPC treatment.

Introduction

Cancer of the nasopharynx is an epithelial cancer developed in the retro-nasal area in the nasal cavity. It was first documented and reported by Regaud and Schmincke separately in 1921 (Regaud 1921; Schmincke 1921). Nasopharyngeal carcinoma (NPC) is frequently confined at the mucosal lining of the fossa of Rosenmuller (pharyngeal recess), posteromedial to the medial crura of the Eustachian tube opening in the nasopharynx (Sham et al. 1990). Due to the small volume and peculiar anatomical structure of nasal cavity (with curved surface), comprehensive inspection of the nasal epithelium by visual means to locate the primary tumor is difficult (Loh et al. 1991). The tumor could spread in different directions (e.g., submucosally) making it difficult to be detected by endoscopy. NPC is highly proliferative and infiltrating and could result in the obstruction of larynx and pharynx. Due to the close proximity of nasopharynx to the skull base, skull base erosion with intracranial extension and/or involvement of the cranial nerve is also common (Roh 2004). NPC is also known as lymphoepithelioma as the primary tumor is infiltrated with abundant leukocyte (majority T lymphocytes). The infiltrating lymphocytes could be found in together with other immune cells such as eosinophils, dendritic cells, and macrophages at the periphery or inside the tumor mass (Jayasurya et al. 2000). It is suspected that the infiltrating immune cells interact with the cancer cells intimately allowing the tumor to escape from immune evasion (Yip et al. 2009). The primary tumor is small and usually causes no symptoms. Patients often present with bilateral glands enlargement and local/

regional lymph node metastasis. Other symptoms include cranial nerve palsies, diplopia, headache, hearing loss, numbness, otitis media, and trismus (Wei and Sham 2005). If the tumor mass is large, it will lead to epistaxis, nasal obstruction, and discharge. Most symptoms are vague and nonspecific at the early stages. By the time of diagnosis, the patients are usually at the advanced stages leading to poor prognosis and local control rate. Hence, there is a desperate need to develop early diagnosis method to improve the treatment outcome.

Clinical Inspection and Radiation Treatment of Undifferentiated NPC

Endoscopy and endoscopic biopsy is the routine procedure for NPC screening (Sham et al. 1989). Computerized tomography (CT) scans and magnetic resonance imaging (MRI) of the skull base will also be used to localize the extent of tumor in the head and neck regions (Brennan 2006). For histological assessment, biopsy is collected at the suspicious site with aid of fiber optic nasopharyngoscope and local anesthesia. Routine and frequent monitoring of the nasopharynx with endoscopic examination is useful to monitor the disease. However, due to the workload and expenses, continuous monitoring of the high-risk group is practically difficult. For histological presentation, cancer cells will have an increase in nuclear size with prominent nucleoli (Pathmanathan et al. 1995). In addition, loss of nuclear polarity and an increased in nuclei to cytoplasm ratio are commonly observed in the epithelial layers (Pathmanathan et al. 1995). For severe dysplasia, the biopsy is characterized with hypercellularity and an increased thickness of the mucosal epithelium, with the preexisting epithelial cells replaced by abnormal cells throughout most of the epithelium (Pathmanathan et al. 1995). For undifferentiated NPC, radiotherapy is recommended for the mainstay treatment regime as the cancer cells demonstrate high sensitivity to radiation with over 90 % locoregional control rate (Lee et al. 2012). Concurrent chemoradiotherapy (radiation treatment together with the use of chemotherapeutic agents such as cisplatin and fluorouracil) is recommended for advanced disease as the chemotherapeutic agents could control the distant metastasis (Lu et al. 2009; Wee et al. 2005).

The use of Molecular Markers in NPC Diagnosis

NPC in the endemic areas is characterized by its consistent association with the human herpesvirus 4, Epstein–Barr virus (EBV). In 1970, zur Hausen et al. observed that EBV virus DNA was harbored in the biopsies collected from the NPC patients but was absent in other tumor cells. At that point, the host cells of EBV remained controversial as it could possibly be derived from the anaplastic cells or from the lymphoid tissues (zur Hausen et al. 1970). Later, the presence of EBV in the tumor cells was confirmed, and many attempts have been made to employ EBV as a form of molecular marker in NPC detection. EBV is a

double-stranded DNA virus, and the genome was protected within the toroid-shaped protein core and a lipid envelope with virus-encoded glycoprotein spikes (Young and Rickinson 2004). According to the International Agency for Research on Cancer (IARC), EBV is classified as group I carcinogen affirming that EBV has a causal role in human malignancies. The virus was first identified in Burkitt's lymphoma cells in 1964 using electronic microscope and is now known to be associated with other human malignancies including Hodgkin's disease, non-Hodgkin's lymphoma, infectious mononucleosis (IM), lymphoepithelioma-like carcinoma, oral leukoplakia and chronic interstitial pneumonitis in AIDS patients, posttransplant lymphoproliferative disease, gastric adenocarcinoma, as well as nasopharyngeal carcinoma.

To date, it is recognized that the virus was present in virtually all the WHO-2 and WHO-3 NPC cells (Sam et al. 1993). For WHO-1 keratinizing NPC, the causal role of EBV remains unresolved as the virus could only be found in a few numbers of patients. In undifferentiated NPC, the carcinogenic role of EBV in the cancer development is much more established. In the epithelial cells, EBV undergoes two phases of life cycle: the latent infection phase and lytic infection phase. At lytic phase, the virus will express specific proteins including early antigen and viral capsid antigen. The immune response of the host cells will induce elevated antibody titers. In latent infection phase, the EBV will express different proteins including Epstein–Barr nuclear antigens (EBNAs), membrane proteins (LMP1, LMP2A, and LMP2B), and nonpolyadenylated nuclear RNAs (EBERs) in their host cells (Fig. 1) (Kieff 1996). In NPC cells, expressions of EBER, EBNA1, LMP2A, and LMP2B are predominant (Raab-Traub 2002). Rowe et al. characterized the latency phase by

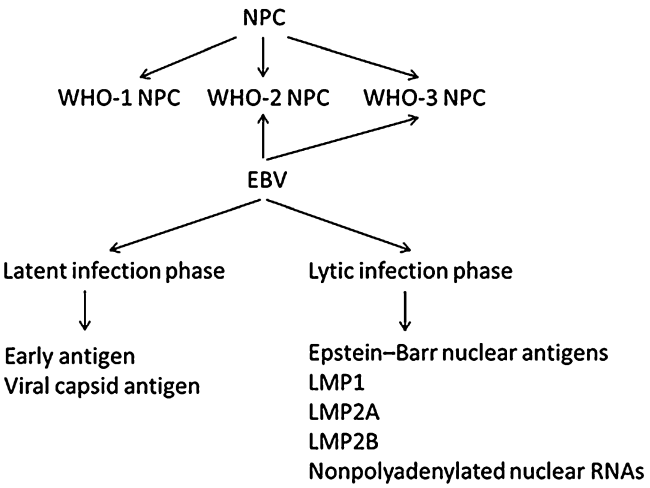

Fig. 1 The association of EBV with WHO-2 and WHO-3 NPC. EBV was present in virtually all the WHO-2 and WHO-3 NPC cells. EBV undergoes two phases of life cycle: the latent infection phase and lytic infection phase. At lytic phase, the virus will express specific proteins including early antigen and viral capsid antigen. In latent infection phase, the EBV will express different proteins including Epstein–Barr nuclear antigens, LMP1, LMP2A and LMP2B, and nonpolyadenylated nuclear RNAs

Table 1 EBV-related biomarkers in NPC

	Type	Detection method	Source of samples
EBER	Noncoding RNA	In situ hybridization	Tissue
EBNA1	Protein	Immunohistochemical staining	Tissue
LMP1	Protein	Immunohistochemical staining	Tissue
EBV DNA	DNA	qPCR	Tissue, blood, saliva
Antibody against EBV antigen	Protein	ELISA	Blood

EBER, EBNA1, LMP1, EBV DNA, and EBV antigens could serve as biomarkers for the diagnosis and prognosis of NPC. EBER, Epstein–Barr-encoded RNA; EBNA1, Epstein–Barr nuclear antigen 1; and LMP1, latent membrane protein 1

the expression patterns of gene products derived from the viral genome (Rowe et al. 1987). For NPC, expression of EBNA1, LMP1, LMP2, EBER, BARTs, and BARF1 in the neoplastic cells was considered as type II latency (Rowe et al. 1987; Fåhraeus et al. 1988; Shibata and Weiss 1992; Gulley and Tang 2008). EBER, EBNA1, LMP1, EBV DNA, and EBV antigens could serve as biomarkers for the diagnosis and prognosis of NPC (Table 1).

Epstein–Barr-Encoded RNA (EBER) 1 and 2 (GenBank Accession Numbers AB065135 and AB065136)

To locate the EBV genome in the biopsy samples, in situ hybridization for EBER remains to be the most efficient and reliable way because EBERs are the most abundantly expressed viral transcripts in the WHO-3 NPC cells and Asian/Chinese ethnicity (Table 2) (Shi et al. 2002). EBERs are thought to be noncoding RNA transcripts (do not code for any protein) as they are nonpolyadenylated (Gulley and Tang 2008). At present, no protein product of EBER is discovered, and the functions of EBER remain unclear. In NPC cells, EBER is transcribed by RNA polymerase III. EBER expression is tumor specific. EBER signals could be detected in the nucleus of the carcinoma cells and are absent in the adjacent normal surface epithelium or the benign stromal cells (Gulley 2001). It is estimated that there are about 1 million copies of EBER transcripts in the nuclei of the latently infected cell (Clemens 1993; Gulley 2001). EBERs are considered as RNA markers for the latently infected cells and are stable in paraffin-embedded tissue sections (Fan et al. 2006). EBER could be detected in all the EBV-associated malignancies except oral hairy leukoplakia (Gilligan et al. 1990). The majority of nasopharyngeal carcinoma patients initially present with enlarged lymph nodes containing metastatic undifferentiated carcinoma of unknown primary. At this circumstance, detection of EBER expression will be useful in identifying the squamous cell-positive lymph node with the squamous cell originating from the NPC (Gulley 2001). As EBER is used as

Table 2 Comparison of diagnostic values of EBV-related biomarkers in NPC

	Abundance	Mode of expression	Samples	Comparison with other biomarkers
EBER	1 million copies per cell	Tumor specific	Paraffin-embedded tissue	The most efficient and reliable way
EBNA1	Not determined	Tumor specific	Paraffin-embedded tissue	High sensitivity and specificity
LMP1	Not determined	Tumor specific	Paraffin-embedded tissue	Low sensitivity
EBV DNA	6,200 copies/mL plasma	Tumor specific	Plasma, serum, nasal brush, saliva	High sensitivity and specificity
Antibody against EBV antigen	Not determined	Not specific to NPC	Blood	Low sensitivity and specificity

EBERs were the most efficient and reliable biomarkers in tissue due to their highest abundance. The diagnosis value of circulating EBV DNA as a noninvasive biomarker for NPC has been demonstrated by a systematic review. EBER, Epstein–Barr-encoded RNA; EBNA1, Epstein–Barr nuclear antigen 1; and LMP1, latent membrane protein 1

Table 3 Prognostic values of EBV-related biomarkers in NPC

	Prognostic values	Samples
EBER	Patients with EBER-positive NPC had a better survival rate after radiation treatment	Paraffin-embedded tissue
EBNA1	High EBNA1 expression in the nasal swab obtained from NPC patients after radiation therapy is a prognostic factor for early and local recurrence	Paraffin-embedded tissue
LMP1	Detection of LMP1 expression in the nasopharyngeal biopsies obtained from postradiation treatment patients could also be regarded as an indicator of early local recurrence	Paraffin-embedded tissue
EBV DNA	The circulating EBV DNA level is a prognostic factor for NPC patients subjected to radiotherapy and is closely associated with the progression-free and overall survival	Plasma, serum, saliva
Antibody against EBV antigen	Measuring the changes in antibody level is only useful in a subset of patients with recurrence or metastases after radiotherapy	Blood

Given the detection of EBV DNA in plasma, serum, and saliva, EBV DNA is particularly useful for periodic monitoring of NPC patients. EBER, Epstein–Barr-encoded RNA; EBNA1, Epstein–Barr nuclear antigen 1; and LMP1, latent membrane protein 1

an indication of NPC cells, in situ hybridization of EBER has been used to monitor NPC patients prospectively after external radiotherapy and is used as a marker for monitoring treatment efficacy (Nicholls et al. 1996). Generally, patients with EBER-positive NPC had a better survival rate after radiation treatment as EBV-positive NPC had a good response rate to radiotherapy (Table 3) (Yip et al. 2006). However, at present, the underlying mechanisms remain poorly understood.

EBNA1 (Epstein–Barr Nuclear Antigen 1, GenBank Accession Number CAD53427)

EBNA1 is a DNA-binding protein and is expressed in all the EBV-associated cancers (Levitskaya et al. 1995; Westhoff Smith and Sugden 2013). It is also expressed in all the EBV-infected NPC cells (Gulley 2001). EBNA1 functions as transcription activator in enhancing EBV gene expression and is essential in maintaining the latently infected status and EBV genome in the host cells (Table 4) (Westhoff Smith and Sugden 2013). The expression level of EBNA1 is closely correlated with the tumor burden (Kottaridis et al. 1996). In addition, EBNA1 expression is essential for the persistent infection of EBV in the host cells as it could prevent the presentation of viral antigen on major histocompatibility complex (MHC) class I molecules and protect the virus from being recognized by the immune cells such as cytotoxic T lymphocyte (Levitskaya et al. 1995). Immunohistochemical staining for EBNA1 protein could be used in screening biopsies obtained from the nasal cavity and regional metastatic lymph node (Table 2). The EBNA transcript could be used as a molecular marker in screening the nasopharyngeal swabs obtained from the NPC patients. Together with the transcript of another EBV gene, LMP1, a sensitivity of 91.4 % (64/70) and a specificity of 98.3 % (348/354) could be reached (Hao et al. 2004). High EBNA1 expression in the nasal swab obtained from NPC patients after radiation therapy is a prognostic factor for early and local recurrence (Table 3) (Hao et al. 2004). EBNA staining is also useful in detecting the cancer cells from the fine-needle aspirate of the regional metastatic lymph node using exfoliative cytologic analysis (Chan and Huang 1990).

Latent Membrane Protein 1 (GenBank Accession Number X58140)

LMP1 is characterized by its transforming properties in the cell lines model (Wang et al. 1985; Kaye et al. 1993). Inhibiting LMP1 expression can trigger cell cycle arrest in NPC cells and could enhance the susceptibility to chemotherapeutic agent

Table 4 Biological functions of EBV-related biomarkers in NPC

	Biological functions	Mechanisms
EBER	No protein product of EBER is discovered, and the functions of EBER remain unclear	Unclear
EBNA1	EBNA1 functions in enhancing EBV gene expression and is essential in maintaining the latently infected status and EBV genome in the host cells	Functions as transcription activator
LMP1	LMP1-expressing NPC cells are phenotypically aggressive	Regulates cell cycle and apoptosis

Targeting LMP1 expression is a promising therapeutic strategy for NPC treatment as expression inhibition of LMP1 could increase the radiation sensitivity of the EBV-infected cells. EBER, Epstein–Barr-encoded RNA; EBNA1, Epstein–Barr nuclear antigen 1; and LMP1, latent membrane protein 1

(Mei et al. 2007). Structurally, LMP1 is a constitutively active homologue of TNFR CD40 receptor. Thus, it will have similar functions in the expressing cells leading to the activation of antiapoptotic genes (Dawson et al. 2012). LMP1-expressing NPC cells are phenotypically aggressive (Table 4) (Busson et al. 2004). Statistical evidence suggested that expression of LMP1 is closely linked to the advanced disease (Zhao et al. 2012). Cases with LMP1 expression exhibited higher cumulative metastatic rates than those without LMP1 expression (Zhao et al. 2012). However, in terms of diagnostic value, LMP1 is not sensitive in comparison with other EBV markers (Table 2). LMP1 is expressed in 50–80 % NPC samples (Zhao et al. 2012). In comparison with EBNA1, LMP1 is less sensitive in detecting the cancer cells in the nasal swabs (Hao et al. 2004). Samples without LMP1 staining may still have strong EBER expression suggesting that LMP1 is not expressed universally in all the EBV-infected cells (Gulley 2001). Targeting LMP1 expression however is a promising therapeutic strategy for NPC treatment as expression inhibition of LMP1 could increase the radiation sensitivity of the EBV-infected cells (Abdulkarim et al. 2003). Detection of LMP1 expression in the nasopharyngeal biopsies obtained from postradiation treatment patients could also be regarded as an indicator of early local recurrence (Table 3) (Hao et al. 2004).

EBV DNA and Viral Load

EBV is a DNA virus (genome size: 173-kb DNA), and the DNA is detected in the nucleus of all the undifferentiated NPC cells (Gulley 2001). The use of in situ hybridization in localizing the tumor cells in the biopsy is not recommended as ERER will offer better sensitivity as a matter of fact that EBER has a higher copy number in the NPC cells (Gulley 2001). EBV DNA could be detected in the peripheral blood of NPC patients with high sensitivity and specificity (Chan and Lo 2002). In a study measuring the plasma EBV DNA concentration, high circulating EBV DNA concentration was detected in patients with primary NPC (6,200 copies/mL), local recurrent NPC (9,200 copies/mL), and distant metastatic NPC (2,050 copies/mL) (Shao et al. 2004a). Circulating EBV DNA may be useful in continuous monitoring of the high-risk group for the development of sporadic disease (Chan et al. 2013). In the NPC tissues, the EBV DNA level is positively correlated with the ERER1-positivie cells and the staging of the tumor patients suggesting that it is correlated with the tumor load (Shao et al. 2004b). Han et al. performed a systematic review on the diagnostic value of circulating EBV DNA in NPC diagnosis. A total of 1,492 NPC and 2,641 controls are used in generating the pooled diagnostic value. The pooled sensitivity and specificity were 0.73 (0.71–0.75) and 0.89 (0.88–0.90) (Table 2). Plasma EBV DNA, in general, had a better detection sensitivity and specificity in comparison with the serum EBV DNA (Han et al. 2012).

The circulating EBV DNA level is a prognostic factor for NPC patients subjected to radiotherapy and is closely associated with the progression-free and overall survival (Table 3) (Chan et al. 2002). Plasma EBV DNA will rise during the

first week post-radiotherapy. The increase is suggested to be caused by the release of EBV DNA from the radiation-induced chronic cell death (Lo et al. 2000). Afterwards, the circulating EBV DNA level will drop significantly with a median half-life of 3.8 days (Lo et al. 2000). In locally recurrent NPC patients treated with nasopharyngectomy (in which the tumor is removed by surgical means immediately), the half-life of circulating EBV is much shorter (i.e., 139 min) after the removal of tumor (To et al. 2003). After 1 week post-radiotherapy, plasma DNA will become undetectable in all the NPC patients (Shao et al. 2004a). It is suggested that the circulating EBV DNA level is an indicator of residual tumor load of the NPC patients (Chan et al. 2002). EBV will shed its virion from the oral mucosa into the saliva of the host (Gulley 2001). It was discovered that EBV DNA could also be detected in the saliva of 80 % primary NPC patients before treatment (Pow et al. 2011). Saliva EBV DNA level was higher in advanced NPC patients, and the level dropped significantly after treatment suggesting that saliva EBV DNA is a candidate indicator of tumor burden (Pow et al. 2011).

Antibody Against EBV Antigen

Peptide-based anti-EBV antibody ELISA has been recommended for use in NPC detection and monitoring the treatment efficacy (Zeng 1985). This is because persistent high anti-EBV antibody against the EBV lytic phase protein is present in the NPC patients (Chien et al. 2001). It is suggested that reactivation of EBV replication at the mucosal layer of head and neck region precedes the development of NPC leading to the production of antibody responses by the hosts' immune system (Henle and Henle 1976). A broad spectrum of anti-EBV antibody is suggested to be useful for NPC detection. Examples included anti-early antigen (EA) IgA, antiviral capsid antigen (VCA) IgA, anti-EBNA1 IgA, and EBV DNase-specific neutralizing antibody. However, the detection sensitivity is highly varying with different antibody titers, and the detection rate could range from 20 % to 100 % (Chang et al. 2013). One major problem of using ELISA is that the antibody could also be detected in normal individuals and non-NPC tumors, including liver, brain, lung, and bone metastases (Table 2) (Shao et al. 2004a). For anti-VCA IgA, anti-EBNA1 IgA, and EBV DNase-specific neutralizing antibody, it could also be detected in 18 % normal individuals (Pickard et al. 2004). About 50 % of the unaffected high-risk individuals from families in which two or more individuals were affected with NPC had high anti-EBV antibody titer (Pickard et al. 2004). In a continuous follow-up and surveillance program with 1,318 volunteers involved in anti-VCA IgA and circulating EBV DNA screening, combination of the two markers could only identify three patients with NPC at the beginning of the program, among which only one patient was serology positive (Chan et al. 2013). EBV antibody titer in combination with other EBV markers could offer a better performance than being used alone (Adham et al. 2013). For monitoring the patients after radiotherapy, the EBV serology has limited value because of the fact that it will remain high in particular patients and in the group with clinical remission

(Shao et al. 2004a). The use of EBV serology may have some value in confirming the clinical remission after radiotherapy (de Vathaire et al. 1988). Measuring the changes in antibody level is only useful in a subset of patients with recurrence or metastases after radiotherapy (Table 3) (Shimakage et al. 1987).

Conclusion

Detection of EBV-derived biomarkers in tissues and EBV DNA in the peripheral blood of NPC patients opens up the possibility to monitor the disease using molecular markers with high sensitivity (Liu et al. 2011). For periodic monitoring of NPC patients, EBV DNA is particularly useful as it could be done on different samples (such as plasma, serum, nasal brush, and saliva) obtained by noninvasive means. The samples could be collected from the patients periodically for continuous monitoring of the pathophysiological changes (Yang et al. 2006). The close association of circulating EBV DNA with the clinical outcomes of treatment provided new tool to predict the treatment outcome. However, the utility of EBV DNA has its limitation as the circulating DNA is not detectable in all the undifferentiated NPC patients in the endemic area (Shotelersuk et al. 2000). In addition, for WHO-1 NPC, which is not associated with EBV latent infection, the use of ERER staining and EBV DNA is not applicable. At present, most of the biomarkers examined for NPC diagnosis are derived from the EBV virus, and little is known about the suitability using somatic biomarkers originating from the cancer cell. Further studies are warranted to identify complementary biomarkers to overcome the limitation of EBV-based biomarkers in NPC screening and monitoring treatment outcome.

Potential Application to Prognosis, Other Diseases, or Conditions

Besides NPC, EBV was also associated with Burkitt's lymphoma and Hodgkin's lymphoma (Table 5) (Kutok and Wang 2006). EBV infection played a crucial role in the development of Burkitt's lymphoma. EBNA1 regulated the cell growth by

Table 5 The association of EBV with Burkitt's lymphoma and Hodgkin's lymphoma

	Diagnosis	Functional roles
Burkitt's lymphoma	EBV infection was only found in 15–30 % cases	EBNA1 regulated the cell growth by inhibiting apoptosis
Hodgkin's lymphoma	EBV infection was only found in about 40 % cases	LMP1 activated NFκB expression, resulting in the accumulation of genetic events that contribute to the initiation and progression of Hodgkin's lymphoma

In light of that EBV infection was only found in 15–30 % Burkitt's lymphoma cases and approximately 40 % of Hodgkin's lymphoma cases, EBV could not serve as a satisfactory diagnostic biomarker in these diseases. EBNA1, Epstein–Barr nuclear antigen 1; LMP1, latent membrane protein 1

inhibiting apoptosis in Burkitt's lymphoma cells. When EBV infected Hodgkin's lymphoma, EBER, EBNA1, LMP1, and LMP2A were expressed. LMP1 activated NFκB expression, resulting in the accumulation of genetic events that contribute to the initiation and progression of Hodgkin's lymphoma. In light of that EBV infection was only found in 15–30 % Burkitt's lymphoma cases and approximately 40 % of Hodgkin's lymphoma cases, EBV could not serve as a satisfactory diagnostic biomarker in these diseases.

Summary points

- EBER, EBNA1, and LMP1 can be applied for diagnosis of NPC and evaluation of the treatment effects of radiotherapy.
- EBERs were the most efficient and reliable biomarkers in tissue due to their highest abundance.
- The combination of EBNA1 and LMP1 resulted in an elevated sensitivity and specificity in screening the nasopharyngeal swabs obtained from the NPC patients.
- Given the detection of EBV DNA in plasma, serum, nasal brush, and saliva, EBV DNA is particularly useful for periodic monitoring of NPC patients.
- The diagnosis value of circulating EBV DNA as a noninvasive biomarker for NPC has been demonstrated by a systematic review.
- The circulating EBV DNA level is a prognostic factor for NPC patients subjected to radiotherapy and is closely associated with the progression-free and overall survival.
- Circulating EBV DNA is not applicable for all the NPC patients; therefore, biomarkers originating from the human cancer cells are required to circumvent the limitation of EBV-based biomarkers in NPC screening and treatment outcome monitoring.

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