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Original article

Sequence variation of Epstein-Barr virus (EBV) BZLF1 gene in EBV-associated gastric carcinomas and nasopharyngeal carcinomas in Northern China

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Abstract

Epstein-Barr virus (EBV) BZLF1 gene can trigger EBV from latent infection to lytic replicative phase. The functions of BZLF1 are well known, while little is known about its gene polymorphism. In order to elucidate the sequence variations of BZLF1 and its association with malignancies, we analyzed BZLF1 gene in 24 EBV-associated gastric carcinomas, 41 nasopharyngeal carcinomas and 24 throat washing samples from healthy donors in Northern China using PCR-direct sequencing method. Three types and 8 subtypes of BZLF1 were identified. A dominant type BZLF1-A was found in 67 of 89 (75.3%) isolates. Type BZLF1-B was characterized by a common Ala deletion at residue 127, which was detected in 21 of 89 isolates (23.6%). Type BZLF1-C contained only one isolate (GC103), which had the same sequence with the prototype B95-8. Among 3 functional domains of BZLF1 protein, the transactivation domain had most mutations, followed by the bZIP domains (the DNA binding domain and dimerization domain). No prevalence of any subtypes or mutations in the functional domains among three specimen groups was found ($P > 0.05$). Our study indicates that BZLF1 subtypes and amino acid changes in functional domains are not preferentially associated with EBV-associated gastric carcinomas or nasopharyngeal carcinomas in Northern China. BZLF1 gene variations are geographically restricted rather than tumor-specific polymorphisms.

Keywords: Epstein-Barr virus; Gastric carcinoma; Nasopharyngeal carcinoma; BZLF1; Polymorphism

1. Introduction

The Epstein-Barr virus (EBV), a ubiquitous human herpes virus, can cause infectious mononucleosis, and is closely associated with Burkitt's lymphoma, nasopharyngeal carcinoma (NPC), and various malignant and non-malignant diseases [1–3]. Many studies have demonstrated that EBV infection is related with some gastric carcinomas (GC). The EBV infection is found in 80–100% of gastric lymphoepithelioma-like carcinoma cases and 2–16% of common types of gastric adenocarcinoma [4–6]. In Northern China this rate is about 7.0% according to our previous study [7].

EBV has two distinct phases in its life cycle: latent and lytic replication. In latent infection, EBV genomic DNA exists as an episome, replicating only once during S phase [8,9] and partitioning accurately into daughter cells during the mitotic phase [8,10]. In some specific situations, the virus can be switched into the lytic cycle [11]. The initiation of lytic replication process greatly depends on the expression of two EBV immediate-early (IE) genes, BZLF1 and BRLF1, whose protein products (ZEBRA and Rta) function as transcriptional

Abbreviation: AA, amino acid; BL, Burkitt's lymphoma; EBV, Epstein-Barr virus; EBVaGC, Epstein-Barr virus-associated gastric carcinoma; NPC, nasopharyngeal carcinoma; PCR, polymerase chain reaction; TW, throat washing.

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transactivators and induce the lytic cascade of viral gene expression [12–14].

ZEBRA (also called EB1) is a phosphoprotein with 245 amino acids (AAs) sharing similar sequences with C/EBP, c-jun, and c-fos. ZEBRA contains three critical functional domains: the transactivation domain (AA 1–167), the DNA binding domain (AA 178–196) and dimerization domain (AA 197–221) [15]. The role of ZEBRA in the lytic phase of the viral life cycle has been extensively studied. One of its earliest and most critical functions is to activate expression of the transcription factor Rta, product of another IE gene BRLF1 [16]. ZEBRA synergizes with Rta to activate transcription of a subset of early lytic cycle genes, many of which encode proteins required for lytic viral DNA replication [12,17,18]. ZEBRA also plays a distinct role in activating viral lytic DNA replication by binding to the origin of lytic cycle replication (oriLyt) and by interacting with and recruiting viral proteins that are essential for lytic replication [17,19,20].

Although the functions of ZEBRA are well known, very little is known about its polymorphisms. Packham and his colleagues have described the polymorphism of the BZLF1 protein in various EBV isolates, elucidating that it did not appear to correlate with EBV 1 and 2 strains but did show a geographical grouping [21]. However, two other studies have suggested that variations in BZLF1 showed a tumor-specific distribution [22,23]. Some other studies merely detected the sequence variations of BZLF1 and did not analyze its polymorphism [24,25].

A notable feature of EBV-associated malignancies is variation in incidence and the proportion of EBV-positive tumors in different geographic regions [26,27]. It remains unknown whether the EBV gene variations are geographically restricted or tumor-specific polymorphisms. EBV isolates from different areas have sequence and functional differences; therefore the association between EBV variations and malignancies has been the subject of intense research in EBV carcinogenesis. In China, the studies on EBV gene polymorphisms were focused on NPCs in the Southern China, the NPC endemic areas. The populations in the NPC non-endemic areas have not been well determined, particularly EBV-associated gastric carcinoma (EBVaGC) patients. In order to explore the association between sequence variations and EBV-associated diseases in Northern China, we analyzed the distribution of BZLF1 gene subtypes as well as variations of functional domains in Northern Chinese EBVaGCs, NPCs and throat washings (TWs) from healthy donors.

2. Materials and methods

2.1. Specimens, cells and DNA extraction

The study was approved by the Medical Ethics Committee at the Medical College of Qingdao University, China. The study group comprised of 62 (69.7%) males and 27 (30.3%) females, with ages from 26 to 78. All the samples (patients and healthy donors) were collected in the same areas. GCs and NPCs specimens were obtained from seven county hospitals of Shandong Province in Northern China, a non-endemic area of NPC. EBV infection in GC and NPC tissues was determined by EBV-encoded small RNA (EBER) 1 using in situ hybridization method as described previously in Ref. [28]. The cases of EBVaGCs and EBV-positive NPCs were used for the study. Throat washings (TWs) were collected from the healthy donors from the same district. The EBV-positive TWs were determined by the BamHI W fragment positive signals using PCR with a BamHI W specific primer pairs [29]. The B95-8 cell line was used as a source of the prototype EBV genome.

DNA extraction from paraffin-embedded tumor tissues was performed using QIAamp DNA FFPE Tissue kit (QIAGEN GmbH, Hilden, Germany), and from TWs by using the standard method with proteinase K digestion and phenol–-chloroform purification.

2.2. Amplification of DNA

The first round polymerase chain reaction (PCR) was performed in a total volume of 25 μl containing 1 × PCR reaction buffer, 100 ng of genomic DNA, 0.5 μM of each primer, 200 μM of each deoxyribonucleotide triphosphates, and 1U Pfu Taq polymerase (TaKaRa Biotechnology Co., Ltd., Kyoto, Japan). PCR amplification was started with an initial denaturation at 94 °C for 5 min. 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min were then performed. A final elongation step at 72 °C for 10 min was also conducted. Specific oligonucleotide primers flanking the BZLF1 gene were designed for nested PCR (Table 1). Primers BZLF1-1 combined with BZLF1-2 (splice1), BZLF1-4 with BZLF1-5 (splice2) as the outer primers. When necessary, 2 μl of the PCR product was used for a second round of PCR, by using internal primers: BZLF1-1 combined with BZLF1-3(splice1), BZLF1-6 with BZLF1-7(splice2). The PCR products were analyzed by electrophoresis through a 1.2% agarose gel.

2.3. Sequencing analysis of PCR products

PCR products were purified using a gel extraction kit (QIAEX II; QIAGEN GmbH, Hilden, Germany), in conditions specified by the manufacturer. PCR amplified fragments were sequenced by means of a Prism ready reaction Dyedeoxy terminator cycle sequencing kit (Applied Biosystems, Foster, USA).

Table 1
Sequences and coordinates of primers used in PCR and sequencing.

Primers	Sequences(5′–3′)	B95-8 coordinates
Splice1		
BZLF1-1	GATGGCGGTAAACAATGG	102072–102089
BZLF1-2	CCTGCTCCTGAGAATGCT	102918–102901
BZLF1-3	CCTCAACCTGGAGACAAT	102816–102799
Splice2		
BZLF1-4	TGCGGACAAAAATCAGGC	102343–102360
BZLF1-5	AAGGGGAGATGTTAGACAGGTA	103227–103206
BZLF1-6	GATTCTTGTATCGCTTTAT	102485–102503
BZLF1-7	GGAGATGTTAGACAGGTA	103223–103206