BAMHI DNA Fragment H-Polymorphism of Epstein–Barr Virus is Associated with the Mutations Present in an 89 BP Sequence Localized in EBNA2 Gene

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Abstract. To characterize the genotypes of Epstein–Barr virus (EBV) isolate present in North Africa, viruses were isolated from B-lymphoblastoid cell lines established from the saliva of both Algerian Nasopharyngeal Carcinoma (NPC) patients and EBV-positive normal individuals, Algerian Burkitt’s lymphoma cell lines, and NPC biopsies. By nucleotide sequence analysis, we showed that there were two specific missense mutations in an 89 bp region of EBNA2 gene at position 49390–49479 of the EBV genome: a mutation at 49449 (C→A) and another mutation at 49444 (T→C), changing their amino acid sequence. The first mutation was found in all B cell lines established from the saliva and 50% of BL cell lines, as well as the W91 cell line, while the second mutation was found in EBV isolates from NPC biopsies, BL cell lines and the M-ABA isolate. A PCR–RFLP analysis on the BamHI DNA fragment H showed that the H1-H2-polymorphism was specifically associated with M-ABA-like mutation, while H-polymorphism was linked with W91-like mutation. The latter was not identified in NPC biopsies, but was found rather in saliva from NPC patients, normal individuals and BL cell lines. The M-ABA-like mutation, on the other hand, was found in 100% of NPC biopsies and some BL cell lines. This suggests that EBV with H1-H2-polymorphism is tightly implicated in NPC development in North Africa rather than EBV with H-polymorphism.

Key words: BL, EBNA2, Epstein–Barr virus isolates, Nasopharyngeal Carcinoma, H polymorphism

Introduction

Epstein–Barr virus (EBV) is a ubiquitous herpes virus, which presents as a widespread infection in the majority of human populations [1,2]. EBV is an etiologic agent of infectious mononucleosis (IM) and is implicated in the pathogenesis of a number of human malignancies of lymphoid or epithelial origin, including Nasopharyngeal Carcinoma (NPC), endemic Burkitt’s lymphoma (BL), Hodgkin’s lymphoma (HL), non-Hodgkin’s lymphoma (NHL), some T-cell lymphomas. It is also implicated in lymphoproliferative diseases occurring in immunosuppressed individuals [3–5]. Approximately 20% of sporadic BL was also associated with the virus in the Caucasian population [3]. In contrast to BL, NPC is tightly associated with EBV [6,7] and its incidence is high in Southern China, Indonesia, and Malaysia, intermediate in North Africa including Algeria, Morocco and Tunisia, and low in other countries [1]. Almost all NPC tumors contain EBV genome and express several proteins encoded by EBV [8–13].

Two major EBV isolates, A and B types, have been identified so far according to their sequence divergence in the EBNA2 gene [14–17], and there are differences in the biologic properties between

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these two types. EBV-A type shows more efficient transforming activity in vitro than EBV-B type [18], and is predominantly found in divers EBV-associated diseases [14–16,19,20,22,23]. EBV-B type, on the other hand, is found in the biopsies from Alaskan NPC, HL [24] or in the pathologic lesions of oral hairy leukoplakia commonly frequent in human immunodeficiency virus-1 (HIV-1) infected individuals [25,26]. Co-infection with the two types of virus has been also reported in HL and NHL of Algerian patients [24]. A restriction-fragment-length polymorphism (RFLP) analysis was recently developed to better characterize the EBV isolates. Among the isolated viruses, three major RFLP are well known: BamHI–F, BamHI–H, and BamHI–W′ polymorphism, representing either an additional BamHI site present in the fragments, resulting in a small “f” for BamHI–F, and two H1 and H2 fragments for BamHI–H or a loss of BamHI site for W′ and I′ fragments resulting in only one BamHI–W′I′ fragment. We have previously demonstrated that EBV isolates associated with North Africa NPC showed the F/H1 + H2/W′I′ type [22,27] and Chinese NPC isolate showed the f/H/W′I′ type [20,21,28,29]. North African NPC EBV differs therefore significantly from Chinese NPC EBV. In addition to the above-mentioned polymorphism, the LMP1 gene contains a polymorphism of a 30 bp deletion in the C-terminal, and this polymorphism is frequent in Chinese NPC biopsies [30,31]. However, some data suggest that this deletion is not limited to NPC [32]. A polymorphism of BZLF1 gene was also reported in NPC-derived EBV isolates [33].

We particularly interested EBNA2 gene localize on BamHI DNA fragment H, because EBNA2 plays a crucial role in B-cell immortalization by EBV, most probably by its ability to transactivate several cellular and viral genes: LMP1, LMP2, CD21, CD23, Bcl2 and c-fgr (3). Mostly importantly, EBNA2 exerts its transactivating function through interaction with Recombination signal binding protein (RBP-Jk) and Notch family protein, so that it could play an important role in cell malignancies (Kieff, 2002). Eighty-nine nucleotide sequence localized in EBNA2 gene was studied in this report, because this domain is not only important for interaction with RBP-Jk protein, but also shows high polymorphism between EBV A and B type (which is used frequently for identification of two EBV types). In our previous report, we found that an 89 bp sequence of EBNA2 gene (frequently used for identification of A or B type EBV) amplified from NPC biopsies hybridized with A-type probe, but the 89 bp sequence amplified from B cell lines established from the saliva of both Algerian NPC patients and individual failed to hybridize with the same probe [27]. We therefore examined the 89 bp nucleotide sequence amplified from Algerian NPC biopsies and saliva-derived B cell lines in comparison with those of known A- and B-types EBV. Since the H1 + H2-polymorphism was specifically found in NPC biopsies and H-polymorphism found rather in saliva from NPC patients, HL and from normal individuals [27], we also examined here whether the H1 + H2-polymorphism is really specific to North African NPC. EBV genomes were isolated from Algerian BL cell lines, B-cell lines established from saliva from normal individuals or NPC patients, and NPC biopsies. EBV genotype studies were carried out using DNA sequencing, PCR and RFLP analysis. In regard to this report and our previous data, three major types of EBV isolates seem to be present in North Africa: EBV B-type [24], M-ABA-like A-type [34] and W91-like A-type EBV [35]. The M-ABA-like mutation was found not only in NPC biopsies, but also in some BL-derived B cell lines, while W91-like mutation was found in B cell lines established from saliva and BL-derived B cell lines. Our study revealed that the H1 – H2-polymorphism is closely related to the M-ABA-like mutation, and H-polymorphism to the W91-like mutation. Moreover, M-ABA-like EBV isolate is closely linked with NPC development in this area. Our finding significantly indicates the different abilities of distinct EBV isolates of subtype A in tumorigenesis.

Materials and Methods

Clinical Samples

Nine NPC biopsy specimens (0.2–0.5 g) were obtained from Mustapha Hospital, Alger, Algeria and immediately snap frozen in liquid nitrogen and stored at −70°C [36]. Tumors were classified as either undifferentiated (WHO stage III) or poorly differentiated (WHO stage III). Clinical staging of