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RESEARCH HIGHLIGHT

A novel Epstein-Barr virus subtype associated with nasopharyngeal carcinoma found in South China

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Nasopharyngeal carcinoma (NPC) is a unique malignancy closely associated with Epstein-Barr virus (EBV) infection and with a restricted geographical distribution [1]. According to a recent World Health Organization (WHO) report, there were around 129,000 new NPC cases reported worldwide in 2018 with greater than 70% of cases in South China and Southeast Asia [2]. In Hong Kong and Guangdong in South China, NPC incidence is as high as 12.8-25.0/100,000 per year [1, 2].

Known risk factors for NPC include a family history of NPC, genetic susceptibility, EBV infection, and environmental factors such as smoking and salted fish consumption [3]. However, previous studies have found that several traditionally accepted risk factors are at most weakly and perhaps not causally associated with contemporary NPC risk [1, 3]. In addition, host genetic factors explain less than 15% of the phenotypic variance [1, 3]. Thus, the cause of NPC endemicity remains enigmatic.

EBV was the first human tumor virus to be discovered. Originally found in the tumor cells of Burkitt lymphoma, a common childhood B-cell malignancy in sub-Saharan Africa. EBV has subsequently been implicated in the etiology of predominantly B-cell lymphomas (particularly in immunosuppressed individuals) as well as carcinomas such as NPC and gastric adenocarcinomas [4]. The tropism of EBV for B lymphocytes and epithelial cells reflects the natural history of normal virus infection whereby EBV establishes lifelong persistence in the memory B cell population of healthy individuals while occasionally replicating in the epithelial cells of the naso- and oro-pharynx [4]. The success of EBV's adaption to the human host is evidenced by this being the most common and persistent virus infection in humans, with approximately 95% of the world's adult population sustaining an asymptomatic life-long infection [4].

The association of EBV infection with NPC was suggested when epidemiological studies found elevated levels of serum antibodies against EBV-encoded lytic cycle antigens in NPC patients [4]. Further serological analysis showed an association between EBV antibody titers and NPC tumor stage and identified viral capsid antigen (VCA)-specific immunoglobulin A (IgA) levels as a useful prognostic marker [4]. A more recent work has identified circulating cell-free EBV DNA as a gold standard biomarker that can be used to stage NPC patients, provide prognostic information, and even be used for early screening of NPC in Hong Kong [5]. Molecular biological studies confirmed the presence of EBV genomes in the tumor cells of almost all endemic NPC tumors with a unique pattern of virus latent gene expression [4]. These observations not only suggested that EBV plays an important role in the pathogenesis of NPC but also raised a series of key questions: How can such a ubiquitous virus infection contribute to the development of a geographically-restricted cancer? Are there any specific EBV strains or subtypes related to NPC development in endemic regions?

There has been much conjecture about the possible contribution of EBV strain variation to the development of NPC

Abbreviations: BALF2, BamHIA Leftward Reading Frame 2; BZLF1, BamHIZ Leftward Frame 1; EBER, Epstein-Barr virus-encoded small RNA; EBNA1, Epstein-Barr virus nuclear antigen 1.; EBV, Epstein-Barr virus; NPC, nasopharyngeal carcinoma; VCA, virus capsid antigen.

and other EBV-associated cancers. As the technology for capturing and sequencing the EBV genome has been improved, a number of studies have shown that small variations across the virus genome may result in functional differences that influence the development of NPC [6, 7]. However, definitive population-based case-control studies of EBV strain variation in NPC patients as compared with the healthy population have been lacking. A recent study published in *Nature Genetics* entitled 'Genome sequencing analysis identifies high-risk Epstein-Barr virus subtypes for nasopharyngeal carcinoma' by Xu et al. [8] addressed this issue, using large-scale EBV whole-genome sequencing to examine EBV subtypes in an attempt to explain the unique NPC endemicity in South China. The authors isolated 215 EBV strains from patients diagnosed with EBV-associated cancers (including NPC, gastric carcinoma, and lymphomas) and 54 from healthy controls from both NPC-endemic and non-endemic regions of China. Through a comprehensive and systematic association analysis of EBV genomic variation and subsequent replication analysis in independent cases and controls, they identified two non-synonymous EBV variants within the *BALF2* gene (BamHIA leftward reading frame 2 encoding a single strand DNA binding protein associated with EBV replication) strongly associated with the risk of NPC (odds ratio [OR] = 8.69 for SNP162476_C and OR = 6.14 for SNP163364_T). Individuals infected with an EBV strain carrying both of these coding variants, EBV subtype BALF2_CCT, have 11 times higher risk for developing NPC than the carriers of low-risk subtype BALF2_ATC. Around 80% of the NPC cases in the Cantonese population carry this high-risk EBV subtype. In addition, evolutionary analysis of BALF2_CCT revealed a unique origin in Asia, followed by clonal expansion in NPC-endemic regions. Currently, over 40% of individuals in South China are infected with this high-risk EBV strain.

This study confirms the critical role of EBV infection in the pathogenesis of NPC and provides an explanation for the striking epidemiological distribution of this tumor in the Cantonese population. By identifying the strongest known NPC risk factor to date, the study heralds the possibility of effective intervention programs where individuals carrying high-risk EBV subtypes could be identified and subsequently monitored as a screening strategy to reduce the disease burden of this common cancer in South China. Furthermore, primary prevention through the development of vaccines against high-risk EBV strains could greatly reduce the disease burden of NPC.

Other recent EBV sequencing studies have highlighted variation in the EBV genome that may have functional consequences impacting the development of virus-associated diseases, including cancer and chronic active EBV infection [6, 7]. Another case-control study of NPC in Hong Kong has identified high-risk EBV subtypes with polymorphisms in the EBV-encoded small RNA (EBER) locus [9]. Whether this reflects real differences in high-risk EBV strains in different populations remains to be determined, and it is possible that the high prevalence of NPC seen in other Southeast Asian countries/regions (e.g., Malaysia and Indonesia) will be associated with different EBV subtypes. The findings of Xu et al. [8] also raise the possibility that different EBV genetic variants may play an instrumental role in the development of other EBV-related malignancies, including certain lymphomas and gastric carcinomas. Comparative analysis of the growing repository of EBV strain sequences from different anatomical sites, populations, and diseases will provide important insights into the natural history of EBV infection and how this can contribute to disease.

The precise role of EBV in NPC carcinogenesis remains obscure. One hypothesis is that NPC-associated EBV strains or subtypes are functionally different from lymphoma-derived strains. Recent studies have found sequence differences in the EBV genome in NPC in the promoter region driving expression of the BamHIZ leftward frame 1 (BZLF1) [10], the immediate early protein responsible for the switch from latent to lytic infection, and in the Epstein-Barr virus nuclear antigen 1 (EBNA1) protein [11], both of which appear to promote EBV replication. These observations are consistent with those from a study on an NPC-derived EBV strain (M81), which was found to be more lytic and to have enhanced tropism for epithelial cells [12]. Thus, it appears that the EBV strain present in NPC may be more replication-competent than previously considered and that other factors (e.g., genetic changes, epigenetic changes, and stem cell environment) might restrict the ability of the virus to replicate in pre-malignant nasopharyngeal epithelial cells. The presence of a more replicative form of EBV in NPC may explain why elevated antibody titers to late viral antigens have diagnostic and prognostic value in this tumor [13] and is consistent with the detection of lytic cycle antigens in NPC [14]. Xu et al. [8] demonstrated that regulation of the EBV lytic cycle is an important factor in the development of NPC and highlighted the need for biological studies to determine the precise mechanistic impact of EBV strain variation. It is now clear that EBV strain variation does have a role in the pathogenesis of NPC, and this provides exciting opportunities for the development of novel therapeutic and diagnostic approaches as well as possibilities for population screening and prophylactic vaccination.

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