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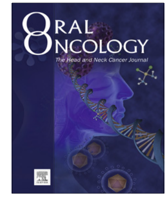

Review

The other side of the coin: Leveraging Epstein–Barr virus in research and therapy

Fenggang Yu [a,*], Wei Jian Tan [b], Yanan Lu [a], Paul A. MacAry [b], Kwok Seng Loh [c]

[a] Department of Otolaryngology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[b] Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[c] Department of Otolaryngology-Head and Neck Surgery, National University Health System, Singapore

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ABSTRACT

Epstein-Barr virus is (EBV) a ubiquitous virus prevalent in 90% of the human population. Transmitted through infected saliva, EBV is the causative agent of infectious mononucleosis (IM) and is further implicated in malignancies of lymphoid and epithelial origins. In the past few decades, research efforts primarily focused on dissecting the mechanism of EBV-induced oncogenesis. Here, we present an alternate facet of the oncovirus EBV, on its applications in research and therapy. Finally, discussions on the prospective utilization of EBV in nasopharyngeal carcinoma (NPC) diagnosis and therapy will also be presented.

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Introduction

Epstein–Barr virus (EBV) is the first human tumor virus discovered 52 years ago based on the electron microscopy observation of virus particles from Burkitt's lymphoma (BL) biopsies [1]. It belongs to human herpesvirus family and infects more than 90% of the world population [2]. EBV exhibits dual tropism, infecting both B lymphocytes and epithelial cells. While infection with the virus during childhood is usually asymptomatic, EBV infection in an adolescent can manifest as infectious mononucleosis (IM) [3,4]. In immunocompetent individuals, the replication of EBV-infected B cells is kept in check by T cell immunity, driving the virus into dormancy. Nonetheless, the virus is capable of persisting in the human population by restricting expression of viral products to EBV-encoded small RNAs (EBERs) and viral microRNAs (miRNAs), establishing latency 0 profile in the memory B cell pool [5].

As an opportunistic human pathogen, EBV was proposed to be involved in B cell malignancies observed in post-transplant lymphoma and AIDS-related lymphoma when the delicate balance between the host immunity and the EBV-infected B cells is perturbed [6–8]. Unlike EBV-associated lymphoid malignancies, the link between EBV and epithelial malignancies is less clear. Although the virus transforms and immortalizes B cell upon infection, the virus does not readily infect epithelial cells, putting forth the contribution of host genetics and environmental factors in EBV-associated NPC. Therefore, it is believed that EBV establishes latency aberrantly in epithelial cells that have already undergone pre-malignant genetic changes [9] as an essential initiation step in the development of NPC. In this article, we will discuss the utility of targeting EBV gene products, the viral episome, and whole virus for research, screening, diagnostic, and future treatment of NPC (Fig. 1).

EBV gene product-ZEBRA

EBV ZEBRA protein (product of gene BZLF1) is a basic leucine zipper transcriptional activator required for latent to lytic reactivation [10,11]. Apart from its intrinsic function in activating EBV lytic cycle, ZEBRA was shown recently to cross the cell membrane and accumulate in the nucleus of lymphocytes. Specifically, a minimal domain (MD11) consisting of 43 amino acids peptide within the ZEBRA protein was found to be efficient in delivering high molecular weight proteins across the lipid bilayer of the cell membrane via direct, non-endocytosis-dependent translocation [12]. As demonstrated by Marchione et al., the utilization of MD11 permitted high-efficiency (70–100%) delivery of fully biological active cargo proteins within two hours with almost no toxicity. Indeed, using MD11, apoptosis of melanoma and colorectal tumor cells can be observed upon the successful delivery of eIF3f (f subunit of the eukaryotic initiation factor 3) across the cell membrane [13]. Taken together, such EBV peptide-based delivery system presented here could represent a potentially powerful tool in cancer treatment, by facilitating the delivery of coupled therapeutics into cancer cells.

* Corresponding author.
E-mail address: entyf@nus.edu.sg (F. Yu).

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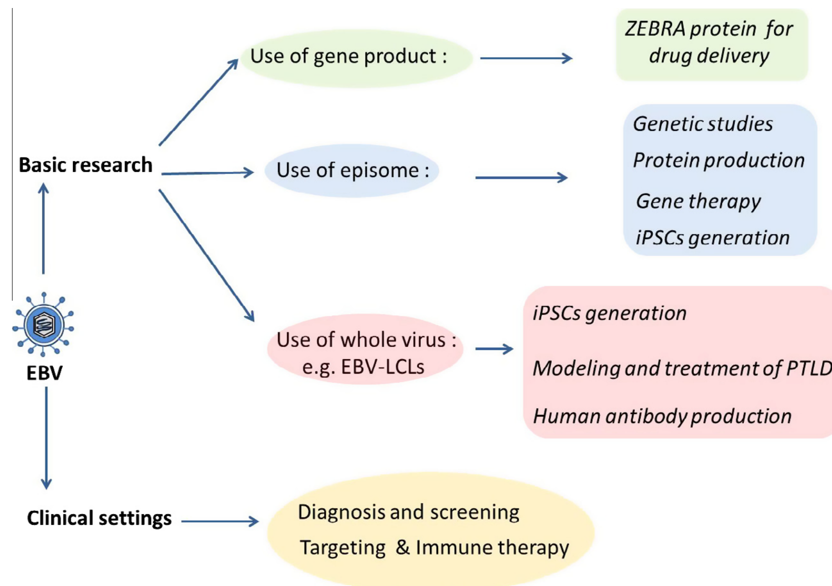

Fig. 1. Applications of EBV in basic research and clinical settings.

EBV episome

EBV episomal vectors were first described by Yates et al. in 1985 [14]. It is comprised of a latent origin of plasmid replication (oriP) and its trans-activating protein EBV nuclear antigen-1 (EBNA-1). Structurally, oriP consists of the family of repeats (FR) element and the dyad symmetry (DS) element, both containing multiple consensus sequences for EBNA-1 binding. While DS serves as the site for the initiation of replication, FR functions as an anchor point for EBNA-1 binding to ensure equal partitioning of viral episome into daughter cells. As a result, episomal vectors replicate once per cell cycle in synchrony with the host chromosomes as extra-chromosomal entities [15]. Given the non-integrative characteristic of the EBV episome and its ability to accommodate large transgene insertion, the use of EBV episomal vector had been extended to genetic studies, protein production, gene therapy, and iPSCs generation.

Employment of EBV episomes in genetic studies

Prior to the establishment of next–generation sequencing, the study of the human genome was technically challenging as the human genome is inherently large. To overcome this problem, the EBV episome was utilized as the shuttle vector to house large fragments of human genome sequences with size ranging from 60 to 330 kb [16]. The high insert capacity of EBV episomes allows the delivery of intact genomic DNA loci to achieve physiological levels of transgene expression. These constructs known as human artificial episomal chromosomes permit the preservation of the human sequences from deletion, recombination, rearrangement, and facilitated the transfer and recovery between expression systems of different organism. The use of EBV episomes had greatly expedited the process of physical mapping and functional identification of human genes.

Employment of EBV episomes for mammalian protein production

In the production of recombinant protein, a stable cell line capable of chromosomal expression of the transgene is ideal but rare [17]. While the use of viral vectors such as retrovirus, adenovirus, and baculovirus can ensure stable expression, these viruses have to be modified to prevent uncontrolled replication in case of accidental release. Apart from the laborious and technical challenges in generating these recombinant viruses, random integration of the transgene into the host chromosome with the use of these recombinant viruses can confound transgene expression and impact recombinant protein production.

In this regard, the use of EBV episome can circumvent the erratic transgene expression and recombinant protein production as the result of random integration. Episomal vectors persist in multiple copies per cell, resulting in amplification of transgene and higher protein expression in a relatively short period of time. Indeed, rapid and high transgene expression had been achieved with the use of EBV episomes in the absence of drug selection [18–20]. Coherent with the excellent capacity in accommodating large transgene, the expression of 185 kb human β-globing transgene had been reported with the stable and sustainable expression for 3 months [20]. Even though high transgene expression can also be achieved with the use of plasmid-based vectors with constitutive promoters such as SV40, CMV, and EF1 promoters, sustainability of the plasmid is always a concern as plasmid often gets diluted upon cell division.

Leveraging on the binding interaction between EBNA-1 protein and EBV episome, further improvements to achieve fast and high yield recombinant protein had been attempted. The enhancing transgene expression in EBV episomal vector is probably attributed to the binding of EBNA-1 to oriP to activate transcription of the transgene, and the oriP enabling nuclear import of transgene [21]. With the establishment of the mammalian HEK293-EBNA-1 cell line (HEK293E), where parental HEK293 cells stably express EBNA-1, the episomal expression of the transgene resulted in a threefold increase of protein yield [22–24]. As reported by Backliwal et al., the utilization of HEK293E combined with high-efficient polyethyleneimine-based transfection resulted in a remarkable efficiency of exceeding 1 g/l of recombinant protein [25]. Notebly, production of recombinant protein at the scale of 100 L with 293E adapted suspension culture had been reported by Philippe et al. [26,27] appealing to industry production for biotechnology.

Gene therapy

Gene therapy is a therapeutic approach to complement a deficient or to correct a defective gene via the introduction of a

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