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Review

The biology of EBV infection in human epithelial cells

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ABSTRACT

EBV-associated human malignancies may originate from B cells and epithelial cells. EBV readily infects B cells *in vitro* and transforms them into proliferative lymphoblastoid cell lines. In contrast, infection of human epithelial cells *in vitro* with EBV has been difficult to achieve. The lack of experimental human epithelial cell systems for EBV infection has hampered the understanding of biology of EBV infection in epithelial cells. The recent success to infect human epithelial cells with EBV *in vitro* has allowed systematic investigations into routes of EBV entry, regulation of latent and lytic EBV infection, and persistence of EBV infection in infected epithelial cells. Understanding the biology of EBV infection in human epithelial cells will provide important insights to the role of EBV infection in the pathogenesis of EBV-associated epithelial malignancies including nasopharyngeal carcinoma and gastric carcinoma.

1. Background

The association of EBV infection with human malignancies has long been recognized [1,2] and could be dated back to the 1960s with the identification of EBV infection in Burkitt's lymphoma, a common childhood cancer in Africa [3]. In addition to Burkitt's lymphoma, EBV infection is also associated with Hodgkin's disease, gastric carcinoma and nasopharyngeal carcinoma (NPC) [4]. In poorly differentiated or undifferentiated NPC, which are the common histopathological types of NPC among southern Chinese, EBV infection is detected in most if not all the NPC cells. However, events regulating EBV entry and maintenance of persistent infection in human epithelial cells, not to say nasopharyngeal epithelial cells, have not been clearly defined [5]. Investigations into the biology of EBV infection in human epithelial cells have been greatly hampered by the difficulty in infecting human epithelial cells with EBV *in vitro*. This contrasts greatly with the ease of infecting B lymphocytes *in vitro*, which readily drives them to proliferation resulting in the establishment of lymphoblastoid cell lines (LCLs). As a result, the biology of EBV infection in human epithelial cells is much less understood compared to that of B lymphocytes. EBV infection of B lymphocytes in human adults results in a state of lymphoblastoid proliferation known as infectious mononucleosis [6]. In immune competent patients, the lymphoblastoid proliferation will eventually subside resulting in establishment of life-long infection of EBV in host memory B cells. The epithelial compartment is believed to play a major role in lytic replication of EBV and has been postulated to be crucial in the shedding of infectious EBV particles into saliva for viral transmission [7]. EBV genes expressed during latent and lytic infections alter the biological properties of infected cells and have been implicated in human carcinogenesis. Understanding the biology of EBV infection in human epithelial cells is crucial to the understanding of the pathogenic role of EBV in human epithelial malignancies including gastric and nasopharyngeal carcinomas.

2. EBV gene expression in infected human epithelial cells

EBV readily infects B lymphocytes in culture *via* the CR2 receptor, which is commonly expressed in B lymphocytes but not in human epithelial cells [8]. In contrast, EBV infection of human epithelial cells *in vitro* is of much lower efficiency. The cell surface receptors of human epithelial cells for EBV infection have not been clearly defined. Recent studies indicated that integrins at the cell surface are involved in mediating EBV infection in human epithelial cells [9,10]. Despite the difficulty involved in infecting human epithelial cells *in vitro*, EBV genome as well as EBV gene expression are readily detected in poorly differentiated or undifferentiated NPC [11,12]. The profile of EBV gene expression in NPC is characteristic of type II latent infection including expression of EBER, EBNA1, LMP1, LMP2, and other EBV genes. The EBV-encoded BARF1 is also commonly expressed in NPC [13,14]. The expression of EBV-encoded BART mRNA and the recent identification of the EBV-encoded microRNAs in NPC further contribute to the complexity of biology of EBV infection and the role of EBV in epithelial malignancies [15,16]. Interestingly, genetic alterations including deletion and methylation of p16 and RASSF1 loci could be identified

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in premalignant nasopharyngeal epithelium prior to EBV infection [17]. These observations raised a crucial question needed to be addressed in NPC pathogenesis *i.e.* whether existence of genetic alterations in premalignant nasopharyngeal epithelium predispose them to EBV infection.

EBV infection of human epithelial cells was achieved in early studies *via* ectopical expression of the CR2 receptor in SV40-immortalized keratinoctyes (SVK) and well-differentiated squamous carcinoma cells (SCC12F) [18,19]. Expression of EBV genes including EBER and EBNA1 was detected in these EBV-infected epithelial cells, which mimicked EBV gene expression in NPC. Interestingly, the lytic EBV gene, BZLF1, was also detected at the early phase of EBV infection of SVK-CR2 cells. Evidence of lytic replication of EBV was also observed when these EBV-infected epithelial cells were stimulated to undergo epithelial differentiation by serum starvation or treatment with a phorbol ester (TPA), suggesting a regulatory role of epithelial differentiation in induction of lytic EBV infection. However, the number of EBV-infected cells, as evidenced by EBNA1 expression, progressively declined when the cells were further propagated in culture. It was, however, not clear if the loss of EBV-infected cells was the result of loss of EBV genome in infected cells or loss of EBV-infected cells in culture. Nonetheless, stable clones of EBV-infected SVK-CR2 cells were established and maintained over long periods of time (>1.5 year). Interestingly, these stable clones of EBV-infected SVK cells were resistant to induction of epithelial differentiation and unable to undergo lytic EBV replication upon stimulation by phorbol ester. These results have two major implications: (a) The EBV gene expression is much more restricted in EBV-infected epithelial cells (predominantly EBER and EBNA1) compared to EBV-transformed lymphoblastoid cell lines, which express a full spectrum of latent EBV genes including EBER, EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, LMP1, LMP2, *etc.*, which are characteristics of type III latent infection. Clearly the differences in host transcription factors in epithelial cells and B cells have major impact on regulation of EBV gene expression in infected cells. (b) The failure of stable clones of EBV-infected cells to undergo lytic infection indicated that loss of epithelial differentiation may be a precondition for EBV to establish stable latent infection in infected cells which has implication on the histological types of EBV-associated NPC, which are predominantly poorly differentiated or undifferentiated in nature. Loss of epithelial differentiation was not observed immediately after EBV infection, but was only observed in stable EBV-infected clones after prolonged propagation, suggesting that EBV infection *per se* was not responsible for the loss of differentiation properties in infected cells.

3. IgA/Sc complex-mediated EBV infection of human epithelial cells

The route of EBV entry into human epithelial cells remains a major focus of investigation. Early study proposed that the polymeric IgA against EBV viral capsid proteins may mediate the entry of EBV into epithelial cells. Polymeric IgA is commonly present in human saliva [20]. The J (joining) chain of the IgA could bind to the secretory component (SC protein) to facilitate the endocytosis-transfer of EBV into epithelial cells. The SC protein is a transmembrane protein reported to be expressed on the basolateral surfaces of epithelial cells and the pseudostratified columnar epithelium and intermediate squamous epithelium covering the lateral nasopharyngeal walls including the epithelial linings of the Fossa of Rosenmüller, which are common sites for development of NPC [20]. Using an HT29 colonic adenocarcinoma cell line cell model, the authors were able to infect these cells using IgA purified from patients with infectious mononucleosis. Eighteen hours after EBV infection of epithelial cells, BZLF1 and early antigen (EAD) could be detected in 4% of cells by immunofluorescence. EBV DNA in infected cells could be detected by PCR analysis. Hence, formation of EBV/IgA/Sc complex could mediate EBV entry *via* an endocytic route into epithelial cells and may represent a physiological route of EBV entry, particularly at the basolateral surfaces of epithelial cells. Subsequent study also reported the presence of SC complex in NPC cells [21]. EBV infection of established NPC cell lines was achieved by this mode of infection [22]. It is well-known that EBV-positive PC cell lines established in culture readily lost their EBV genomes upon prolonged propagation. Using the anti-EBV IgA fraction prepared from sera of NPC patients to mediate EBV entry into epithelial cells, re-infection of these EBV-negative NPC cells with EBV was achieved [22]. EBV DNA and expression of EBV genes including EBER and EBNA1 was detected in these re-infected NPC cells. Up-regulation of mRNA expression of EGFR, TGF-alpha, IL-1 beta, IL-6, and GMSCF was observed within 1 to 2 weeks after EBV infection, representing responses of host cells to EBV infection. However, the EBV genomes in the infected NPC cells were not stable and were rapidly lost in subsequent passages within 3–4 weeks [22]. Apparently, EBV infection did not confer *in vitro* proliferative advantage to the infected-NPC cells. However, a faster growth rate of EBV-infected NPC cells and a more invasive histological phenotype were observed when they were injected into athymic nude mice, suggesting a selective growth advantage of EBV-infected cells *in vivo* [23]. The nature of this selective growth advantage is unclear but may involve enhanced expression of angiogenic factors and expression of MMP2/9 detected in EBV-infected NPC cells [22].

4. Cell type tropism for EBV infection

Apparently, EBV employs different entry mechanisms to infect human B lymphocytes and epithelial cells. The entry of EBV into B lymphocyte requires interaction of the viral envelope glycoprotein, gp350/220, with the CR2 receptor of B lymphocytes and the binding of a complex of three viral glycoproteins, gH, gL and gp42 with the HLA antigen class II on the surface of B lymphocytes [24–26]. Entry of EBV into B lymphocytes requires fusion of virus envelope and cell membrane of B lymphocyte. Fusion of virus envelope with cell membrane of the B lymphocyte is triggered by gp42. In contrast, EBV fusion with epithelial cells is impeded by gp42. Interestingly, EBV virions released from B lymphocytes, are deficient in gp42 which renders them more efficient to infect epithelial cells, but less efficient to infect B lymphocytes. Upon egression from infected epithelial cells, the gp42 is reloaded onto the viral capsule and EBV regains the high infection efficiency for B lymphocytes. This change in cell type tropism for EBV infection suggests that EBV shuttles between epithelial cells and B-lymphocytes in humans during its infection cycle [26]. This observation supports a role of pharyngeal epithelial cells in lytic EBV infection and shedding of infectious EBV particles for transmission.

5. Cell-to-cell contact facilitates EBV infection of human epithelial cells

A major breakthrough in EBV infection of human epithelial cells was reported in 1998 by directly co-culturing the EBV-producing lymphoblastoid cells (Akata cells) with epithelial cells [27]. The efficiency of EBV infection was greatly improved by this cell-to-cell contact method of infection. There was however a great variability in EBV infection rate among different cell lines examined. The highest infection rate was observed in NU-GC-3 (human gastric adenocarcioma), DLD-1 (human colon adenocarcioma), and 293 (adenovirus E1-transformed human embryonic kidney cells). Expression of EBNA1 was uniformly detected in