Epstein–Barr Virus

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Epstein–Barr virus is a ubiquitous persistent virus, which is involved in the development of some human cancers. A licensed vaccine to prevent Epstein–Barr virus infection is lacking. BamHI-A rightward frame 1 is a viral protein specifically detected in both nasopharyngeal and Epstein–Barr virus-positive gastric cancers. It has been proposed that this viral protein confers cancer properties to infected epithelial cells and is involved in the escape of cancer cells from immune recognition.

Keywords: Epstein–Barr Virus,BARF1,epithelial cells

1. Introduction

The human gammaherpesvirus-4 (HHV-4), commonly referred to as Epstein–Barr virus (EBV), is a member of the Herpesviridae family and Lymphocryptovirus genus [1]. EBV establishes a latent persistent infection affecting more than 90% of the human population worldwide [2]. Primary EBV infection in children usually occurs without any symptoms. Conversely, during adolescence and early adulthood primary EBV infection may produce infectious mononucleosis (IM) disease, which is characterized by an IgM antibody response against EBV, the circulation of increased loads of latently infected B-cells, and the development of EBV-specific CD8 + T cells [3]. The circulating CD8 + T cells recognizing lytic EBV antigens are detected approximately five days after the appearance of IM symptoms and are responsible for the specific immune response against EBV-infected cells [4][5].

In 2018, an estimated 200,000 newly diagnosed cancers were related to EBV infection [2][6], including both lymphoid and epithelial malignancies. According to the International Agency for Research on Cancer (IARC), only three epithelial tumors (nasopharyngeal, gastric, and lymphoepithelial carcinomas) have proved to be undoubtedly associated with EBV infection [7][8][9]. On the other hand, this virus has been found in tumors of the oral cavity, breast, and uterine cervix, among others, which indicates the need for further investigation. Among the EBV proteins involved in the malignant transformation of epithelial cells, the BamHI-A rightward frame 1 (BARF1) is of utmost importance [10]. This lytic gene is highly expressed in nasopharyngeal carcinomas (NPC) and EBV-associated gastric (EBVaGC) carcinomas during latency [11][12], but is virtually undetectable in B-cells and lymphomas, in which it can mostly be found during the viral lytic cycle [13][14]. This fact allows for the consideration of BARF1 as an epithelial-specific EBV oncogene as well as an attractive potential therapeutic target for EBV-associated epithelial tumors [15]. Previously, the therapeutic potential of BARF1 has been extensively reviewed [16].

2. Contribution of BARF1 expression to epithelial cell carcinogenesis and impaired host immune response

2.1 BARF1 and Cell Proliferation Rates

BARF1 interacts with some cell cycle-regulating proteins, promoting epithelial cell proliferation. In HaCaT cells, BARF1 was demonstrated to increase the expression of cyclin D1 at transcriptional and protein levels [17]. Additionally, increased cell proliferation was evidenced in EBV-negative cells stably transfected with a BARF1 encoding vector [10]. Notably, in gastric cancer (GC) cells, transfection with the BARF1 gene promoted a reduction in p21WAF1 expression [18], suppressing one of the most important regulatory mechanisms of cell proliferation. BARF1 also promotes cell proliferation by increasing NF-κB RelA and upregulating the microRNA-146a-5p, which in turn downregulates SMAD4 [10]. The inactivation of SMAD4, a critical mediator of the growth-inhibiting TGFβ signaling pathway, reduces the expression of some CDK inhibitors (e.g., p15, p21, and p27), resulting in uncontrolled cell proliferation [19].

2.2. Anti-Apoptotic Effects of BARF1

BARF1 protects epithelial cells from the intrinsic cell death pathway by regulating anti-apoptotic (e.g., Bcl-2, Bcl-xL) and pro-apoptotic (e.g., Bax) pathways. Transfection of primary epithelial and NPC cells with the BARF1 gene induces increased Bcl-2 levels [12]. Similarly, Bcl-xL upregulation was evidenced in HaCaT BARF1-transfected cells when compared with BARF1-negative control cells [17]. Another study reported the capacity of BARF1 to protect GC cells from apoptosis by increasing the Bcl-2/Bax ratio [20]. The increase in the Bcl-2/Bax ratio was similarly evidenced in GC cells expressing BARF1 after Taxol (paclitaxel) exposure. Likewise, a significant reduction in the percentage of these cells showing late apoptosis events (nuclear fragmentation) was evidenced in the same conditions [20], suggesting a potential contribution of BARF1 to apoptosis-based therapy resistance in EBVaGC.

2.3. Immortalization and Tumorigenic Properties of BARF1

Telomere elongation by the telomerase enzyme is a prerequisite by which cells can reach unlimited replicative potential and also contributes to tumorigenic properties [21]. Increased telomerase activity was reported in BARF1-transfected epithelial cells, which was comparable to that obtained in human telomerase reverse transcriptase (hTERT)-transfected cells, allowing these cells to escape from senescence [12]. In the same study, it was demonstrated that hTERT activation in BARF1-transfected cells is accompanied by c-Myc upregulation [12], suggesting a potential synergism between BARF1 and c-Myc to induce hTERT activation. On the other hand, BARF1 was able to induce anchorage-independent growth in soft agar as well as altered migration of HEK-293 cells [22]. Furthermore, the infection of NPC cells with EBV carrying the BARF1 gene induced tumor growth in nude mice, but not in EBV-infected cells lacking BARF1 [23]. Altogether, these results suggest a central role for BARF1 in the tumorigenicity of NPC and GC cells in vivo, although other factors are required for malignant transformation.

2.4. BARF1 Expression and Modulation of Host Immune Response

BARF1 also contributes indirectly to epithelial carcinogenesis by promoting evasion of both innate and adaptive immune responses. This viral protein is responsible for the sequestration of the macrophage colony-stimulating factor (M-CSF, also known as CSF-1), inducing a disruption in the differentiation and activity of macrophages [24][25]. For instance, the hijack of M-CSF by sBARF1 induces a reduction in the expression of a variety of macrophage differentiation-specific markers such as CD14, CD11b, CD16 and CD169 [26]. This fact also interferes with the function of mononuclear cells, by inhibition of interferon-alpha (IFN-α) production and release [27]. Moreover, M-CSF pre-incubation with sBARF1 inhibited M-CSF receptor, Akt, and MAPK phosphorylations in myeloid leukemia cells, which attributes a role of BARF1 in the survival and proliferation capacity of macrophages [26].

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