MicroRNA in Epstein–Barr Virus-Associated Cancers

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A conservative estimate suggests that almost 1.4 million of malignancies are associated with oncogenic viruses, including the hepatitis B virus, hepatitis C virus, Kaposi sarcoma-associated herpesvirus, human T lymphotropic virus type 1, human papillomaviruses, and Epstein–Barr virus (EBV). The oncogenic properties of these viruses are directly related to their ability to activate processes needed for cellular proliferation, survival, migration, and immune evasion. Among these viruses, EBV, formerly designated as the human herpesvirus type 4 (HHV-4), is a y-herpesvirus containing a linear, double-stranded DNA genome of ~172 kilobase pairs (kbp), encoding nearly 80 proteins and 46 functional small untranslated RNAs. The genetic material of EBV is enclosed in an icosahedral nucleocapsid surrounded by the viral tegument and lipid-containing outer envelope. EBV is transmitted through oral contact, particularly in the early years of life, usually without causing disease. EBV can also be transmitted through organ transplantation and blood transfusion. The life cycle of EBV primarily involves the infection of lymphocytes and potentially epithelial cells. Although EBV often exists as an asymptomatic infection, it is involved in the development of about 1.5% of all cancers worldwide. In fact, EBV was the first virus to have been directly associated with cancer in humans. EBV-associated neoplasms affect both immune-competent and immunocompromised hosts, including, for example, some organ transplant recipients. Immune dysregulation and genetic susceptibility are probable co-factors in most, if not all, EBV-associated cancers.

Keywords: microRNAome ; miRNA ; EBV ; immune evasion ; carcinogenesis ; classical Hodgkin’s lymphoma ; Burkitt lymphoma ; diffuse large B-cell lymphoma ; nasopharyngeal carcinoma ; gastric carcinoma

1. Overview

Epstein–Barr virus (EBV) is associated with a variety of malignancies. In this review, we discuss EBV-encoded microRNAs and ncRNAs and consider how their detection could aid in the diagnosis, prognostication, and monitoring of treatment in patients with EBV-associated malignancies, including classical Hodgkin’s lymphoma (cHL), Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), nasopharyngeal carcinoma (NPC), and gastric carcinoma (GC).

EBV is a direct causative agent in around 1.5% of all cancers. The oncogenic properties of EBV are related to its ability to activate processes needed for cellular proliferation, survival, migration, and immune evasion. The EBV latency program is required for the immortalization of infected B cells and involves the expression of non-coding RNAs (ncRNAs), including viral microRNAs. These ncRNAs have different functions that contribute to virus persistence in the asymptomatic host and to the development of EBV-associated cancers. In this review, we discuss the function and potential clinical utility of EBV microRNAs and other ncRNAs in EBV-associated malignancies. This review is not intended to be comprehensive, but rather to provide examples of the importance of ncRNAs.

2. Epstein–Barr Virus

In 2018, an estimated 2.2 million infection-attributable cancer cases were diagnosed worldwide. A conservative estimate suggests that almost 1.4 million of these were associated with oncogenic viruses [1], including the hepatitis B virus, hepatitis C virus, Kaposi sarcoma-associated herpesvirus, human T lymphotropic virus type 1, human papillomaviruses, and Epstein–Barr virus (EBV) [2]. The oncogenic properties of these viruses are directly related to their ability to activate processes needed for cellular proliferation, survival, migration, and immune evasion [3]. Among these viruses, EBV, formerly designated as the human herpesvirus type 4 (HHV-4), is a *y*-herpesvirus containing a linear, double-stranded DNA genome of ~172 kilobase pairs (kbp), encoding nearly 80 proteins and 46 functional small untranslated RNAs [2][4]. The genetic material of EBV is enclosed in an icosahedral nucleocapsid surrounded by the viral tegument and lipid-containing outer envelope. EBV is transmitted through oral contact, particularly in the early years of life, usually without causing disease [5]. EBV can also be transmitted through organ transplantation and blood transfusion [6]. The life cycle of EBV primarily involves the infection of lymphocytes and potentially epithelial cells. Although EBV often exists as an asymptomatic infection, it is involved in the development of about 1.5% of all cancers worldwide [7]. In fact, EBV was the

first virus to have been directly associated with cancer in humans. EBV-associated neoplasms affect both immune-competent and immunocompromised hosts, including, for example, some organ transplant recipients. Immune dysregulation and genetic susceptibility are probable co-factors in most, if not all, EBV-associated cancers [8].

The EBV life cycle begins when the virion enters naïve B-lymphocytes, or in some cases, perhaps memory B-lymphocytes, probably following initial infection of epithelial cells [9]. Although the exact mechanisms of EBV entry into epithelial cells are becoming clearer, how the virus crosses the epithelial barrier to infect B cells in vivo remains unknown [10]. The virus may infect the epithelial cells, replicate, and then be released to infect B cells in the underlying areas, but there is no direct evidence for this and normal epithelial cells appear to be resistant to infection from the apical (i.e., mucosal) side [11]. If the virus is unable to infect epithelial cells directly, then it may be able to traverse the epithelial membrane barrier to access B cells, perhaps when the epithelial lining is damaged, or becomes leaky during inflammation [12]. Conversely, on exit to the oropharynx, there is some evidence that transfer infection of a virus from B cells to epithelial cells can occur via the basolateral surface [12].

Following cell entry, the viral genome is released into the nucleus where it becomes circularized, an event that maintains the EBV genome as an extrachromosomal episome that is readily replicated and is used as a marker of viral clonality [13]. Furthermore, the EBV genome carrying few epigenetic tags associates itself with histones and becomes methylated due to the similarity of its nucleosomal structure with that of the host genome. DNA methylation and histone modification are vital epigenetic mechanisms that regulate gene expression necessary for completing the viral life cycle [14]. To ensure its persistence in infected B cells, EBV enters the latency phase resulting in the silencing of some viral genes, an event that is crucial for evading host cell immunity [9]. There are different latency states. For instance, expression of the latency III or the ‘growth program’ consisting of six EBV nuclear antigens (EBNA-1, -2, -3A, -3B, -3C, -LP) and three latent membrane proteins (LMP-1, -2A, -2B) results in the proliferation and immortalization of primary B cells [15]. Latency II or the ‘default program’ consisting of EBNA1, LMP1, LMP2A, and LMP2B, is expressed in EBV-infected germinal center B cells. The latency I program, which is limited to the expression of only one protein, EBNA1, is responsible for maintaining viral episomes in dividing memory B cells [13]. Latency 0, characterized by the absence of viral gene expression, is observed in non-dividing memory B cells [9]. During these different latency programs, the virus utilizes non-coding RNAs, including viral microRNAs [16]. Latency is halted and viral reactivation begins when memory B cells terminally differentiate to plasma cells. The new virions are then released from B cells and may infect epithelial cells where the virus is amplified for cell-to-cell spread or infection of a new host [17]. Compared to B cells, the nature of EBV infection of epithelial cells is less well understood [18].

The small non-coding, non-polyadenylated RNAs EBER-1 and EBER-2 are also abundantly expressed in both EBV-infected non-malignant and cancerous cells [19]. Owing to the significantly longer half-life of EBER-1, it is usually present at ten-fold higher levels compared with EBER-2 [20]. The EBERs do not code any proteins and their abundance makes them a valuable diagnostic tool for EBV detection; using in situ hybridization, the detection of EBER has been established as the most sensitive and practical method for detecting EBV [21]. However, the precise contribution of the EBERs to the viral life cycle and to malignant transformation remain unclear. The EBERs are not required for EBV-induced transformation of primary B-lymphocytes, but assemble into stable ribonucleoprotein particles with the La and L22 proteins [22][23], and bind the interferon-inducible, double-stranded RNA-activated protein kinase PKR, suggesting that they may be involved in suppressing the antiviral effects of the interferons [24]. EBER2 RNA may regulate the levels of LMP2 and it has been shown to exist in a complex with PAX5; this complex can regulate LMP2A/B and LMP1 expression [25]. Knockdown of EBER2 also decreased EBV lytic replication [26]. In a recent study, EBER2 was shown to be able to substitute for the Marek’s disease virus telomerase RNA-like viral RNA [27].

Apart from its non-coding EBER, EBV can also express a number of microRNAs (miRNAs). miRNAs are highly conserved, small, non-coding RNAs important for gene expression in various organisms, including humans. Although small, miRNAs outnumber coding sequences in the human genome. Their role in gene expression is not limited to normal functions but are also important in the development of disease. Of particular interest, here is the dysregulation of miRNAs in cancer [28][29][30][31]. miRNA genes are transcribed into primary RNA (pri-miRNA) by RNA polymerase II or III; and then cleaved into precursor miRNA (pre-miRNA) by a microprocessor complex comprised of endonuclease enzymes, DROSHA or DGCR8. The pre-miRNA is then transported from the nucleus into the cytoplasm via a nucleocytoplasmic exporter, which contains exportin-5 (XPO5) and RAN-GTP. In the cytoplasm, the pre-miRNA is cleaved into a miRNA duplex by a complex of DICER and transactivating response RNA-binding protein (TRBP), and further cleavage results in the generation of the mature miRNA. The mature miRNA is incorporated within the RNA induced silencing complex (RISC) and Argonaute proteins (Ago2). This protein complex is responsible for regulating translation of the target mRNAs [29][30]. Dysregulation in any step of miRNA biogenesis, for example by genetic, epigenetic, and transcriptional mechanisms, may result in alterations in mRNA translation. For instance, upregulation of oncogenic miRNAs (oncomiR)

(for example, those involved in regulation of the cell cycle), and/or downregulation of tumor suppressive miRNAs (tumor-suppressor miR) can contribute to carcinogenesis [30][32]. Some well-studied oncomiRs include miR-155, which is over-expressed in some types of lymphoma and leukemia [33][34]. Let-7 miRNA is an example of a tumor-suppressor miR, and is downregulated in lung cancer [35]. However, some miRNAs can be oncogenic or tumor suppressive depending upon the type of cancer. For example, miR-17-92 is upregulated in lung cancer [36], but downregulated in breast cancer [37]. Moreover, a single miRNA may be involved in more than one biological pathway [28][29][30][31][32].

Apart from miRNAs, other non-coding RNAs including long non-coding RNAs (lncRNAs) are also increasingly linked to cancer. The first lncRNA shown to be involved in cancer was HOX Antisense Intergenic RNA (HOTAIR), which is upregulated in breast cancer [38]. Another lncRNA, the Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1), is also upregulated in lung and colorectal cancers [39][40].

Due to the observed changes in the expression of miRNAs in cancer, miRNAs in circulating body fluids are being investigated as potential minimally invasive biomarkers that could help in both the diagnosis and monitoring of patients [32][41][42]. In this review, we consider the functions of the EBV-encoded miRNAs and of other ncRNAs, in normal and cancer cells, and highlight their potential clinical utility.

3. Conclusions

The emerging field of ncRNA biology is already providing multiple opportunities, not only to better understand the pathogenesis of malignant disease, but also in the development of new biomarkers of disease, in defining novel therapeutic targets and/or even as alternative therapies. Although EBV was the first human virus identified to express miRNA, challenges remain before applications involving EBV miRNA, or other ncRNA encoded by the virus, can be routinely adopted into clinical practice.

References

1. de Martel, C.; Georges, D.; Bray, F.; Ferlay, J.; Clifford, G.M. Global burden of cancer attributable to infections in 2018: A worldwide incidence analysis. Lancet Glob. Health 2020, 8, e180–e190.
2. de Elgui Oliveira, D.; Müller-Coan, B.G.; Pagano, J.S. Viral carcinogenesis beyond malignant transformation: EBV in the progression of human cancers. Trends Microbiol. 2016, 24, 649–664.
3. Krump, N.A.; You, J. Molecular mechanisms of viral oncogenesis in humans. Nat. Rev. Microbiol. 2018, 16, 684–698.
4. Wang, L.W.; Jiang, S.; Gewurz, B.E. Epstein-Barr virus LMP1-mediated oncogenicity. J. Virol. 2017, 91, e01718-16.
5. Jenson, H.B. Epstein-Barr virus. Pediatr. Rev. 2011, 32, 375–383.
6. Smatti, M.K.; Al-Sadeq, D.W.; Ali, N.H.; Pintus, G.; Abou-Saleh, H.; Nasrallah, G.K. Epstein-barr virus epidemiology, serology, and genetic variability of LMP-1 oncogene among healthy population: An update. Front. Oncol. 2018, 8.
7. Farrell, P.J. Epstein–Barr virus and cancer. Annu. Rev. Pathol. Mech. Dis. 2019, 14, 29–53.
8. Ko, Y.H. EBV and human cancer. Exp. Mol. Med. 2015, 47, e130.
9. Guidry, J.T.; Birdwell, C.E.; Scott, R.S. Epstein–Barr virus in the pathogenesis of oral cancers. Oral Dis. 2018, 24, 497–508.
10. Chen, J.; Longnecker, R. Epithelial cell infection by Epstein–Barr virus. FEMS MicroBiol. Rev. 2019, 43, 674–683.
11. Tugizov, S.M.; Berline, J.W.; Palefsky, J.M. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. Nat. Med. 2003, 9, 307–314.
12. Shannon-Lowe, C.; Rowe, M. Epstein-Barr virus infection of polarized epithelial cells via the basolateral surface by memory B cell-mediated transfer infection. PLoS Pathog. 2011, 7, e1001338.
13. Yin, H.; Qu, J.; Peng, Q.; Gan, R. Molecular mechanisms of EBV-driven cell cycle progression and oncogenesis. Med. MicroBiol. Immunol. 2019, 208, 573–583.
14. Fernandez, A.F.; Rosales, C.; Lopez-Nieva, P.; Grana, O.; Ballestar, E.; Ropero, S.; Espada, J.; Melo, S.A.; Lujambio, A.; Fraga, M.F.; et al. The dynamic DNA methylomes of double-stranded DNA viruses associated with human cancer. Genome Res. 2009, 19, 438–451.
15. Kang, M.S.; Kieff, E. Epstein-Barr virus latent genes. Exp. Mol. Med. 2015, 47.

16. Zhao, M.; Nanbo, A.; Sun, L.; Lin, Z. Extracellular vesicles in Epstein-Barr virus' life cycle and pathogenesis. Microorganisms 2019, 7, 48.

17. Laichalk, L.L.; Thorley-Lawson, D.A. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. J. Virol. 2005, 79, 1296–1307.

18. Reusch, J.A.; Nawandar, D.M.; Wright, K.L.; Kenney, S.C.; Mertz, J. Cellular differentiation regulator BLIMP1 induces Epstein–Barr virus lytic reactivation in epithelial and B cells by activating transcription from both the R and Z promoters. J. Virol. 2015, 89, 1731–1743.

19. Howe, J.G.; Steitz, J.A. Localization of Epstein-Barr virus-encoded small RNAs by in situ hybridization. Proc. Natl. Acad. Sci. USA 1986, 83, 9006–9010.

20. Clarke, P.A.; Sharp, N.A.; Clemens, M.J. Expression of genes for the Epstein-Barr virus small RNAs EBER-1 and EBER-2 in Daudi Burkitt's lymphoma cells: Effects of interferon treatment. J. Gen. Virol. 1992, 73, 3169–3175.

21. Wu, T.C.; Mann, R.B.; Charache, P.; Hayward, S.D.; Staal, S.; Lambe, B.C.; Ambinder, R.F. Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin's disease. Int. J. Cancer 1990, 46, 801–804.

22. Lerner, M.R.; Andrews, N.C.; Miller, G.; Steitz, J.A. Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. Proc. Natl. Acad. Sci. USA 1981, 78, 805–809.

23. Toczyski, D.P.; Matera, A.G.; Ward, D.C.; Steitz, J.A. The Epstein-Barr virus (EBV) small RNA EBER1 binds and relocalizes ribosomal protein L22 in EBV-infected human B lymphocytes. Proc. Natl. Acad. Sci. USA 1994, 91, 3463–3467.

24. Clemens, M.J.; Laing, K.G.; Jeffrey, I.W.; Schofield, A.; Sharp, T.V.; Elia, A.; Matys, V.; James, M.C.; Tilleray, V.J. Regulation of the interferon-inducible eIF-2 alpha protein kinase by small RNAs. Biochimie 1994, 76, 770–778.

25. Gregorovic, G.; Boulden, E.A.; Bosshard, R.; Elgueta Karstegl, C.; Skalsky, R.; Cullen, B.R.; Gujer, C.; Rämer, P.; Münz, C.; Farrell, P.J. Epstein–Barr viruses (EBVs) deficient in EBV-encoded RNAs have higher levels of latent membrane protein 2RNAexpression in lymphoblastoid cell lines and efficiently establish persistent infections in humanized mice. J. Virol. 2015, 89, 11711–11714.

26. Lee, N.; Moss, W.N.; Yario, T.A.; Steitz, J.A. EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. Cell 2015, 160, 607–618.

27. Kheimar, A.; Kaufer, B.B. Epstein-Barr virus-encoded RNAs (EBERs) complement the loss of herpesvirus telomerase RNA (vTR) in virus-induced tumor formation. Sci. Rep. 2018, 8, 209.

28. Calin, G.A.; Croce, C.M. MicroRNA-cancer connection: The beginning of a new tale. Cancer Res. 2006, 66, 7390–7394.

29. Macfarlane, L.A.; Murphy, P.R. MicroRNA: Biogenesis; Function and Role in Cancer. Curr. Genom. 2010, 11, 537–561.

30. Jansson, M.D.; Lund, A.H. MicroRNA and cancer. Mol. Oncol. 2012, 6, 590–610.

31. Lin, S.; Gregory, R.I. MicroRNA biogenesis pathways in cancer. Nat. Rev. Cancer 2015, 15, 321–333.

32. Di Leva, G.; Croce, C.M. miRNA profiling of cancer. Curr. Opin. Genet. Dev. 2013, 23, 3–11.

33. Eis, P.S.; Tam, W.; Sun, L.; Chadburn, A.; Li, Z.; Gomez, M.F.; Lund, E.; Dahlberg, J.E. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc. Natl. Acad. Sci. USA 2005, 102, 3627–3632.

34. Cimmino, A.; Calin, G.A.; Fabbri, M.; Iorio, M.V.; Ferracin, M.; Shimizu, M.; Wojcik, S.E.; Aqeilan, R.I.; Zupo, S.; Dono, M.; et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc. Natl. Acad. Sci. USA 2005, 102, 13944–13949.

35. Yanaihara, N.; Caplen, N.; Bowman, E.; Seike, M.; Kumamoto, K.; Yi, M.; Stephens, R.M.; Okamoto, A.; Yokota, J.; Tanaka, T.; et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006, 9, 189–198.

36. Hayashita, Y.; Osada, H.; Tatematsu, Y.; Yamada, H.; Yanagisawa, K.; Tomida, S.; Yatabe, Y.; Kawahara, K.; Sekido, Y.; Takahashi, T. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005, 65, 9628–9632.

37. Moi, L.; Braaten, T.; Al-Shibli, K.; Lund, E.; Busund, L.T.R. Differential expression of the miR-17-92 cluster and miR-17 family in breast cancer according to tumor type; results from the Norwegian Women and Cancer (NOWAC) study. J. Transl. Med. 2019, 17, 334.

38. Gupta, R.; Shah, N.; Wang, K.; Kim, J.; Horlings, H.M.; Wong, D.J.; Tsai, M.C.; Hung, T.; Argani, P.; Rinn, J.L.; et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010, 464, 1071–1076.

39. Ji, P.; Diederichs, S.; Wang, W.; Böing, S.; Metzger, R.; Schneider, P.M.; Tidow, N.; Brandt, B.; Buerger, H.; Bulk, E.; et al. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003, 22, 8031–8041.
40. Xu, C.; Yang, M.; Tian, J.; Wang, X.; Li, Z. MALAT-1: A long non-coding RNA and its important 3’ end functional motif in colorectal cancer metastasis. Int. J. Oncol. 2011, 39, 169–175.
41. Reddy, K.B. MicroRNA (miRNA) in cancer. Cancer Cell Int. 2015, 15.
42. Kosaka, N.; Iguchi, H.; Ochiya, T. Circulating microRNA in body fluid: A new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010, 101, 2087–2092.