

• Review •

Research advancement on relationship between Epstein-Barr virus and breast cancer

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[Abstract] The etiology of breast cancer is still unclear. The well-known risk factors, including reproductive and other factors affecting circulating sex hormones, and genetic susceptibility, explain only about 50% of all breast cancer incidence. More and more studies have shown interest in the possibility that breast cancer may be caused by viral infection. Epstein-Barr virus (EBV) is one of the candidate viruses, but the association of EBV with breast cancer remains controversial. Here we reviewed the studies on EBV biology and the association of EBV with breast cancer, including EBV detection in breast cancer tissues, serological tests, cytologic experiments and clinical analyses, and described the limitations of current studies and future directions.

Key words: Epstein-Barr virus, breast neoplasm, association

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Breast cancer is the most common malignant tumor in women, with about 1.15 million new cases and 410 000 death annually.¹ Many studies have investigated the etiology of breast cancer, and found that reproductive factors, genetic susceptibility and factors that affecting sexual hormone level in the body are closely related to the occurrence and development of breast cancer. However, these factors could only explain about 50% of all breast cancers.² This prompts the exploration into the etiology of breast cancer via other aspects. Many studies have demonstrated that viral infection is closely associated to the occurrence and development of certain tumors, for example, human papillomavirus (HPV) and cervical cancer, hepatitis B virus (HBV) and hepatic cancer, Epstein-Barr virus (EBV) and Burkitts lymphoma.³ Such associations make scientists wonder about the relationship between breast cancer and viruses.

Early in 1830s, Bittner found that a substance in the breast milk of mice could render increased risk of developing breast cancer for the offsprings via breast feeding.⁴ In subsequent studies, this substance was identified as mouse mammary tumor virus (MMTV). Later, MMTV-like granules were also seen in human breast cancer tissue. But so far, there is no adequate evidence to support that MMTV-like granules are involved in the tumorigenesis of breast cancer. In 1994, Horiuchi et al.⁵ detected EBV in 2 of 3 breast cancer specimens. However, the detection rates of EBV varied

greatly in subsequent studies, which has complicated the relationship between EBV and breast cancer. Herein, we summarized the biological features of EBV and the association between EBV and breast cancer.

Biological features of EBV and associated tumors

EBV, a member of gamma-herpes viruses, is a DNA herpes virus, with a full length of 184 kb in the viral genome, encoding around 100 proteins and with human B lymphocyte tropism first identified by Epstein and Barr in 1964 when they were studying malignant lymphoma in African children.⁶ When B cells are infected with EBV, viral gene will be adequately expressed in very few of these infected cells, where about 80 proteins are synthesized, including early antigen (EA), viral capsid antigen (VCA) and membrane antigen (MA); DNA is replicated as well, and eventually complete virion is constructed and released to achieve proliferative infection; while in most infected cells, the virus induces latent infection without replication, and only expresses 6 nuclear antigens (EBNA1, EBNA2, EBNA3a, EBNA3b, EBNA3c and LP), 3 latent membrane proteins (LMP1, LMP2a and LMP2b) and 2 small RNAs (EBER1 and EBER2) to help the virus escape from host immune surveillance and achieve life-time latent infection.^{7, 8} Based on different antigen expression profiles, latent infections are classified into 4 types: type 0, where main expressed products are LMP2 and 2 EBERs; type I, where main expressed products are EBNA1 and 2 EBERs; type II, where main expressed products are EBNA1, 3 LMPs and 2 EBERs; type III, where all the latent antigens, namely 6 EBNAs, 3 LMPs and 2 EBERs, are expressed.^{7, 9} Under certain circumstance, latent infection of EBV can be transformed into proliferative status, where infected cells are lysed and viral particles are released to achieve dissemination.⁷

EBV is mainly disseminated via saliva, and infects the oropharynx epithelia.⁹ According to statistics, over 90% of adults on the earth are infected with EBV, but the onset age of primary infection varied vastly. In developing countries,

infection occurs at early age, for example, over 80% of 1-year-old children in Uganda were serologically positive for EBV; while in American countryside, only 45% of the counterparts were serologically positive.⁶ Such inconsistency may be driven by the different social and health conditions and subsequently different EBV exposure rates in these countries. Primary infection generally occurs in early childhood and is often asymptomatic as latent infection; if infection occurs later in adolescence or adulthood, it can cause infectious mononucleosis. After primary infection, EBV can parasitize in B cells on a long-term basis as latent infection. Although latent infection is generally asymptomatic, numerous studies have proved that EBV plays important roles in the development of associated tumors.⁶ In 1997, EBV was recognized as class I carcinogen by International Agency for Research on Cancer (IARC). EBV-associated tumors include immuno-suppression associated tumors (such as post-transplantant lymphoproliferative disorder, AIDS-associated lymphoma), B-cell lymphoma (such as Hodgkins lymphoma, Burkitts lymphoma), T-cell and NK-cell lymphoma, and epithelium-derived carcinomas [nasopharyngeal carcinoma (NPC), gastric adenocarcinoma and lymphoepithelial carcinoma].⁹ The detection rates of EBV vary vastly in different tumors (for instance, EBV was detected in over 90% of endemic Burkitts lymphoma, while in less than 25% of gastric adenocarcinoma), indicating that EBV has different oncogenic or co-oncogenic mechanisms in different tumors.^{2,9}

Studies on the association between EBV and breast cancer

EBV detection in cancer tissues. Ever since Horiuchi et al.⁵ used polymerase chain reaction (PCR) to detect EBV in breast cancer tissues in 1994, PCR has been used in many studies to detect EBV; however, the detection rates of EBV varied greatly among different studies. For example, Labrecquet et al.¹⁰ and Zhang et al.¹¹ amplified the EBV repetitive sequence, BamHIW, in 20%-30% of the breast cancer specimens, while Gaffey et al.¹² amplified no

EBNA1 gene in 35 breast cancer specimens. Glaser et al.² believed that the detection rate was related to the target genes of PCR detection, with the highest positive rates for EBER and BamHIW, followed by LMP-1 and EBNA3b, and the lowest rate for EBNA1.

Although the sensitivity of PCR detection is high, it can not tell whether the viral DNA is from tumor cells or infiltrating lymphocytes; on the other hand, in-situ hybridization and immunohistochemical techniques can localize positive staining to identify viral DNA sources. Since EBER is expressed in all types of latent infection, in-situ hybridization of EBER is considered the golden standard in detecting latent infection of EBV.¹³ EBER is highly expressed in various EBV-associated tumors,¹³ but for breast cancer, in-situ hybridization on EBER revealed negative results in most cases, only a few showed positive signals; in addition, these positive signals were from a small portion of the tumor cells.^{10, 14-17} Immunohistochemical assays used EBNA1, LMP and ENBA2 as target antigens to detect EBV in cancer tissues. Likewise, positive results were seen only in part of the tumors, and the positive signals were from breast tumor cells, rather than infiltrating lymphocytes.¹⁸⁻²¹ Furthermore, some studies have used laser micro-dissection technique to separate breast tumor cells from infiltrating lymphocytes and then performed PCR detection in tumor cells to determine whether viral DNA existed in tumor cells. Again, only a few studies detected EBV in some of the tumor cells.^{14, 22} Based on the results described above, EBV exists in some breast cancer cells, rather than lymphocytes.

Besides these qualitative detection methods, some studies have used real-time quantitative PCR to detect viral load in cancer tissues. Murray et al.²³ performed real-time quantitative PCR on 92 breast cancer specimens and obtained positive results in 19 of these specimens; however, the viral load was pretty low (0.1-7.1 EBV copies/1000 cells), while as positive control, the viral load in NPC was more than 2000 EBV copies/1000 cells. Arbach et al.²⁴ amplified EBV genome in 44 (46%) breast cancer tissue samples, but the viral load was similarly low, with the

minimal level of 0.02 EBV copies/1000 cells and the maximal level of 883 EBV copies/1000 cells; only 3 samples showed an load of over 20 EBV copies/1000 cells; after micro-dissection on the samples, only some of the tumor cells maintained positive results, with a viral load of 15-6333 copies/1000 cells. Arbach et al.²⁴ suggested that proliferative infection of EBV might occur in breast cancer.

EBV is seen only in some of the tumor cells and the viral load is pretty low, which places doubt on the association between EBV and breast cancer. But some studies suggested that there might be a lagging effect in EBV infection, that is, EBV infection plays an important role in the occurrence of breast cancer, but gradually disappear during tumor development;¹⁰ it is also possible that infection occurs after canceration of the cells and increases the malignancy of the tumor;^{10, 16} EBV genome might be altered when being integrated into the chromosome of host cells.²⁵ All these uncertain infection mechanisms might be the reason why EBV was detected only in some of the tumor tissues.

Serological studies. Both antigens expressed in EBV proliferative infection phase (such as EA, VCA and MA) and antigens expressed in latent infection phase (such as EBNA and LMP) can trigger immune responses in the body and initiate the production of specific antibodies.²⁶ In the past few decades, immuno-fluorescent or immuno-enzyme labelling methods were often used to detect EBV antibodies in serum, but they were gradually replaced by enzyme-linked immunosorbent assay (ELISA) in recent years.²⁷ Since the infection rate of EBV is high in general population, VCA-IgG, MA neutralizing antibody and ENBA1 antibody may be persistently positive in the serum of healthy carriers.²⁶ Therefore, differentiating the patients with EBV-associated disease from healthy carriers of EBV should be based on the specific antigens in various EBV infection statuses and the antibody levels, rather than on the presence of EBV antibodies in serum. For example, EBV induces type II latent infection in NPC, where EBNA1 and 3 LMPs are highly expressed; in addition, cytolysis of cancer cells is common, and thus many lytic

phase antigens including Zta, EA, VCA and MA are constantly expressed; therefore, the serological profile of NPC is characterized by elevation of numerous EBV antibodies.²⁸ In recent years, ELISA is increasingly used to detect EBNA1-IgA, EBNA1-IgG and Zta-IgG as a screening test of NPC.²⁹ Cheng et al.³⁰ have revealed high levels of EBNA1-IgA, EBNA1-IgG and Zta-IgG in 60% of NPC patients and 0.9% of healthy individuals, while those with high levels of all three antibodies were at extremely high risk of developing NPC (OR=138). In other EBV-associated tumors (such as Hodgkins lymphoma and endemic Burkitts lymphoma), the serological changes is often seen as significant elevation in VCA and EA antibody titers at several months or even years before clinical diagnosis.⁶

Currently, studies on EBV serology in patients with breast cancer are rare. Richardson et al.³¹ determined VCA-IgG in 208 breast cancer patients and 169 controls, and found no significant difference in positive rates between two groups. Angeloni et al.³² determined the antibody of EBV replication-associated protein BFRF1 in the serum of cancer patients and obtained negative results in all the 71 breast cancer patients, but positive results were seen in 31 (78%) NPC patients and 7 (47%) Burkitts lymphoma patients. The infection status of EBV in breast cancer is still unknown. No proper viral protein has currently been selected as the detection target for serological assays. Hence, although a few available studies failed to find out the serological difference between breast cancer patients and healthy individuals, it should not be concluded that EBV is not associated to breast cancer.

Cytological experiments. Speck et al.³³ revealed that mammary epithelial cells could be infected by EBV via direct exposure to EBV-transformed lymphoblasts. Xue et al.³⁴ found that EBV gene p31 stimulated the growth of normal mammary epithelial cells. Lin et al.³⁵ established EBV-infected breast cancer cell lines MCF7 and BT474, and found that EBV enhanced the growth of these breast cancer cells. These studies suggested that EBV could invade mammary epithelium and promote mammary

growth, and might be carcinogenic to a certain extent.

EBV infection and clinical features of breast cancer. A few studies have revealed correlation between EBV infection and the clinical features of breast cancer. For instance, the more malignant the tumor was, the higher the EBV positive rate was; the positive rate of EBV was higher in estrogen receptor-positive tumors than in estrogen receptor-negative tumors;¹⁸ it was higher in tumors with lymph node metastasis than in those without lymph node metastasis.³⁶ However, some other studies suggested that EBV positive rate are not correlated to tumor histological grade, expression of estrogen receptor and lymph node involvement.¹⁹ Fina et al.¹⁵ suggested that viral load in EBV-positive breast cancer specimens in highly prevalent area of NPC was higher than that in lowly prevalent area. Arbach et al.²⁴ found that EBV infection could increase the chemoresistance of breast cancer.

Current issues and future research directions

Since EBV is detected only in some of the tumors, and the detection rates were inconsistent among different studies; in addition, viral load in cancer tissues is extremely low, all these facts make the relationship between EBV and breast cancer complicated and confusing. The huge inconsistency in detection rate among different studies might be mainly driven by the following factors: First of all, no standard experiment methods are established for EBV detection in cancer tissues. For instance, the specimens are processed differently in different studies (formalin-treated sample or fresh cancer tissue), PCR target genes, primers, and cycle conditions are different, and target genes or target antigens for in-situ hybridization and immunohistochemical staining are different as well. Secondly, the infection status of EBV in breast cancer cells is still unclear, thus expressed antigens may be different from those in other EBV-associated tumors. Currently, no proper detection marker has been identified for EBV detection in breast cancer. Lastly, most study designs lack epidemiologic considerations. The

vast majority of the cases were selected from clinical organization, but not based on population sampling. The sample sizes were small in most studies, and the populations were therefore not representative, whereas EBV positive rate might vary among different populations.²

To figure out the relationship between EBV and breast cancer, the first thing in future work is to establish a highly-sensitive and specific EBV detection scheme to figure out the presence of EBV in breast cancer tissues. Besides EBER in-situ hybridization, whether other target sequences or antigens (such as LMP and ENBA) can serve as new detection targets has yet to be proven. Cytological experiments and animal experiments can be used to seek for more evidences on the carcinogenic effect of EBV. Moreover, epidemiologic studies should also be conducted to explore the variation of EBV infection rate and the serological profiles among different breast cancer populations, so as to provide evidences for the association between EBV and breast cancer. Breast cancer is the most common malignant tumor in women; it will be critical implications for the prevention, early recognition and treatment of breast cancer if EBV is identified as a oncogenic or co-oncogenic factor for breast cancer, even if it is responsible for only a small portion of breast cancers.

References

- [1] Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world [J]. *J Clin Oncol*, 2006,24(14):2137-2150.
- [2] Glaser SL, Hsu JL, Gulley ML. Epstein-Barr virus and breast cancer: state of the evidence for viral carcinogenesis [J]. *Cancer Epidemiol Biomarkers Prev*, 2004,13(5):688-697.
- [3] Carrillo-Infante C, Abbadessa G, Bagella L, et al. Viral infections as a cause of cancer (review) [J]. *Int J Oncol*, 2007,30(6):1521-1528.
- [4] Bittner JJ. Some possible effects of nursing on the mammary gland tumor incidence in mice [J]. *Science*, 1936,84(2172):162.
- [5] Horiuchi K, Mishima K, Ohsawa M, et al. Carcinoma of stomach and breast with lymphoid stroma: localisation of Epstein-Barr virus [J]. *J Clin Pathol*, 1994,47(6):538-540.
- [6] Hsu JL, Glaser SL. Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications [J]. *Crit Rev Oncol Hematol*, 2000,34(1):27-53.
- [7] Kutok JL, Wang F. Spectrum of Epstein-Barr virus-associated diseases [J]. *Annu Rev Pathol*, 2006,1:375-404.
- [8] Khanna R, Burrows SR, Moss DJ. Immune regulation in Epstein-Barr virus-associated diseases [J]. *Microbiol Rev*, 1995,59(3):387-405.
- [9] Thompson MP, Kurzrock R. Epstein-Barr virus and cancer [J]. *Clin Cancer Res*, 2004,10(3):803-821.
- [10] Labrecque LG, Barnes DM, Fentiman IS, et al. Epstein-Barr virus in epithelial cell tumors: a breast cancer study [J]. *Cancer Res*, 1995,55(1):39-45.
- [11] Zhang LP, Xu LZ. Detection of Epstein-Barr virus DNA in breast lesions by PCR [J]. *Chin Oncol*, 1997,7(2):79-81. [in Chinese]
- [12] Gaffey MJ, Frierson HF Jr, Mills SE, et al. Medullary carcinoma of the breast. Identification of lymphocyte subpopulations and their significance [J]. *Mod Pathol*, 1993,6(6):721-728.
- [13] Gulley ML. Molecular diagnosis of Epstein-Barr virus-related diseases [J]. *J Mol Diagn*, 2001,3(1):1-10.
- [14] McCall SA, Lichy JH, Bijwaard KE, et al. Epstein-Barr virus detection in ductal carcinoma of the breast [J]. *J Natl Cancer Inst*, 2001,93(2):148-150.
- [15] Fina F, Romain S, Ouafik L, et al. Frequency and genome load of Epstein-Barr virus in 509 breast cancers from different geographical areas [J]. *Br J Cancer*, 2001,84(6):783-790.
- [16] Chu PG, Chang KL, Chen YY, et al. No significant association of Epstein-Barr virus infection with invasive breast carcinoma [J]. *Am J Pathol*, 2001,159(2):571-578.
- [17] Li SY, Li J, Hu JH, et al. Study on gene products of EBV infection in breast carcinoma tissues of patients [J]. *Maternal Child Health Care China*, 2008,23(1):87-88. [in Chinese]
- [18] Bonnet M, Guinebretiere JM, Kremmer E, et al. Detection of Epstein-Barr virus in invasive breast cancers [J]. *J Natl Cancer Inst*, 1999,91(16):1376-1381.
- [19] Preciado MV, Chabay PA, De Matteo EN, et al. Epstein-Barr virus in breast carcinoma in Argentina [J]. *Arch Pathol Lab Med*, 2005,129(3):377-381.
- [20] Yang WT, Xu LZ, Zhang TM, et al. Epstein-Barr virus infection in breast cancer [J]. *J Fudan Univ (Med Sci)*, 1998,25(3):175-178. [in Chinese]
- [21] Trabelsi A, Rammeh S, Stita W, et al. Detection of Epstein-Barr virus in breast cancers with lymphoid stroma [J]. *Ann Biol Clin (Paris)*, 2008,66(1):59-62.
- [22] Thorne LB, Ryan JL, Elmore SH, et al. Real-time PCR measures Epstein-Barr virus DNA in archival breast adenocarcinomas [J]. *Diagn Mol Pathol*, 2005,14(1):29-33.
- [23] Murray PG, Lissauer D, Junying J, et al. Reactivity with A monoclonal antibody to Epstein-Barr virus (EBV) nuclear

- antigen 1 defines a subset of aggressive breast cancers in the absence of the EBV genome [J]. *Cancer Res*, 2003,63(9): 2338–2343.
- [24] Arbach H, Viglasky V, Lefeu F, et al. Epstein-Barr virus (EBV) genome and expression in breast cancer tissue: effect of EBV infection of breast cancer cells on resistance to paclitaxel (Taxol) [J]. *J Virol*, 2006,80(2):845–853.
- [25] Griffin BE. Epstein-Barr virus (EBV) and human disease: facts, opinions and problems [J]. *Mutat Res*, 2000,462(2–3):395–405.
- [26] Rickinson AB, Kieff ED. Epstein-Barr virus [M]. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2007:2655–2700.
- [27] Hu WW, Zong YS, Li FP, et al. Comparison of 6 antibody assays detecting Epstein –Barr virus for serodiagnosis of nasopharyngeal carcinoma [J]. *Chin J Clin Oncol*, 2006,33(14): 795–798. [in Chinese]
- [28] Zhang JX, Chen HL, Zong YS, et al. Epstein–Barr virus expression within keratinizing nasopharyngeal carcinoma [J]. *J Med Virol*, 1998,55(3):227–233.
- [29] Ng MH, Chan KH, Ng SP, et al. Epstein–Barr virus serology in early detection and screening of nasopharyngeal carcinoma [J]. *Ai Zheng*, 2006,25(2):250–256.
- [30] Cheng WM, Chan KH, Chen HL, et al. Assessing the risk of nasopharyngeal carcinoma on the basis of EBV antibody spectrum [J]. *Int J Cancer*, 2002,97(4):489–492.
- [31] Richardson AK, Cox B, McCredie MR, et al. Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study [J]. *Br J Cancer*, 2004,90(11):2149–2152.
- [32] Angeloni A, Farina A, Gentile G, et al. Epstein-Barr virus and breast cancer: search for antibodies to the novel BFRF1 protein in sera of breast cancer patients [J]. *J Natl Cancer Inst*, 2001,93(7):560–561.
- [33] Speck P, Longnecker R. Infection of breast epithelial cells with Epstein-Barr virus via cell-to-cell contact [J]. *J Natl Cancer Inst*, 2000,92(22):1849–1851.
- [34] Xue SA, Lampert IA, Haldane JS, et al. Epstein-Barr virus gene expression in human breast cancer: protagonist or passenger? [J]. *Br J Cancer*, 2003,89(1):113–119.
- [35] Lin JH, Tsai CH, Chu JS, et al. Dysregulation of HER2/HER3 signaling axis in Epstein-Barr virus-infected breast carcinoma cells [J]. *J Virol*, 2007,81(11):5705–5713.
- [36] Fawzy S, Sallam M, Awad NM. Detection of Epstein-Barr virus in breast carcinoma in Egyptian women [J]. *Clin Biochem*, 2008,41(7–8):486–492.