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Epstein- Bar Virus and Cytomegalovirus Infection Association with Breast Cancer.

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Abstract

Cancer is a pathological condition that may be induced by variable factors. Viruses are implicated as a cause of cancer. Breast cancer is common in Iraqi community. Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) are implicated as a cause of breast cancer. In this review we present the association between development of breast cancer and EBV and CMV infections.

Keywords: Epstein-Barr virus, Cytomegalovirus, Breast cancer, EBV, CMV.

1. Epstein Barr Virus (EBV)

1.1 Historical background of EBV Virus

Epstein Barr virus (EBV) has been implicated as a cause of malignant transformation in a number of lymphoid and non-lymphoid cell types. The first association of EBV with cancer was reported in 1964 and coincided with discovery of the virus by Epstein, Achong and Barr in electron micrographs of cells cultured from patients with endemic Burkitt's lymphoma [1]. Epstein Barr Virus was classified as a group1 carcinogen, an indication that there is the strongest possible evidence linking it to human cancer [2].

1.2 Taxonomy and classification

Epstein Barr Virus is grouped as a member of the Herpesviridae family, subfamily gamma Herpesviridae, genus lymphocryptovirus. The Herpesviridae family contain viruses grouped together based on the architecture of their virion [3]. Two major types of EBV strains have been recognized, EBV type-1 and type-2, and they differ biologically and in their geographic and ethnic prevalence's but have no clear differences in EBV associated clinical diseases [4].

1.3 Structural Characteristics

1.3.1 Epstein Barr Virus Particles

EBV virus particle with icosahedral capsid with diameter of 100 to 110 nm and consists of a core containing a linear, double stranded DNA; an icosahedral capsid, approximately 100-110 nm in diameter. Viral capsid is containing 162 capsomeres with a hole running down the long axis; an amorphous, sometimes asymmetric material that surrounds the capsid, designated as the tegument; and an envelope containing viral glycoprotein spikes on its surface [3]. The Epstein-Barr virus surface glycoprotein H (gH) is essential for penetration of B cells but also plays a role in attachment of virus to epithelial cells [5].

1.3.2 Epstein Barr Virus genome

EBV genome is a linear, double stranded DNA, with a length of 170kb. While the genome is circular plasmid in the nucleous of the latently infected cells. At both ends of the linear form of the genome terminal repeat (TR) sequences are present and the infected cells circularization mediated by TR. The unusual large tenderly repeated DNA sequence in EBV genome termed as major internal repeat (IR1). The IR1 site divides the EBV genome into long and short

unique sequences (UL and US) which are filled with closely packed genes [6]. In addition, the EBV genome contains a viral cytokine, vIL-10, that was pirated from the host genome. This viral cytokine can prevent macrophages and monocytes from activating T-cells are required for EBV-dependent transformation of B-cell [7].

1.4 Epstein Barr Virus antigens

Epstein Barr Virus antigens are divided into three classes, based on the phase of the viral life cycle in which they are expressed.

1-Latent phase antigens are synthesized by latently infected cells. These include the Epstein–Barr Nuclear Antigens (EBNAs) and the Latent Membrane Protein (LMPs). Their expression reveals that an EBV genome is present only EBNA1, needed to maintain the viral DNA episomes, is invariably expressed; expression of the other latent phase antigens may be regulated in different cells. LMP1 mimics an activated growth factor receptor [8].

Epstein Barr Nuclear Antigens-1(EBNA-1) was expressed in all actively dividing EBV-infected cells and is responsible for binding the viral episomes through its origin of replication (OriP) to the mitotic cellular DNA, assuring replication and transfer of virus genome to all daughter cells [9]. EBNA-1 was also involved in the transcriptional control of other latency proteins, a function that is independent of episomes maintenance [10].

Expression of EBNA1 in the B cell compartment of transgenic mice leads to development of lymphomas, and thus EBNA1 can be considered a viral Oncogene [11].EBNA1 also interacts with certain viral promoters, and is thereby involved with transcriptional regulation of LMP1 and the EBNAs, including itself [12]. Despite being expressed in all virus-infected cells and capable of raising an antibody response [13], EBNA1 is a relatively poor target for cytotoxic T cells (CTL) [14]. Generally, EBV-positive cells expressing only EBNA1 are resistant to CTL mediated lysis in vitro [15] and CTL responses against EBNA1 are absent in vivo [12].

2-Early antigens are non-structural protein whose synthesis is not dependent on viral DNA replication. The expression of early antigens indicates the onset of productive viral replication [8].

3-Late antigens are the structural component of the viral capsid (viral capsid antigen) and viral envelope (glycoproteins). They are produced abundantly in cells undergoing productive viral infection [8].

1.5. Entry of EBV to cells and life cycle

1.5.1. Cell tropism

Epstein-Barr virus is with distinct lymphocytes cell tropism. Lymphocyte infection is generally found in B cells, and although EBV infection of T and natural killer (NK) cells has been seen it is generally restricted to disease states [16]. There is also evidence for EBV infection of monocytes and macrophages [17]. Circulating infected B cells are rare in healthy carriers, with between 1 and 50 EBV-positive cells per million B cells, and are generally of the memory phenotype, that is surface Ig-positive but surface IgD-negative [18].

Definitive proof for EBV infection of epithelial cells in healthy individuals has yet to be found. Evidence supporting a role for epithelial infection by EBV includes the presence of viral DNA, mRNA and protein in explants cultures of tonsils from healthy seropositive individuals [19], and the presence of EBV genomes in dysplastic epithelial cells adjacent to gastric adenocarcinoma [20]. Demonstrates that EBV is capable of epithelial infection under certain conditions [21]. In vitro, primary epithelial cells are generally EBV resistant, although some cell lines can be infected.

1.5.2.Infection of B cells

Epstein Barr Virus is spread through salivary contact and the virus enters through the epithelium that lines the nasopharynx. Infection of B-cells is initiated by the binding of the major EBV outer envelope glycoprotein gp 350/220 with the cellular complement receptor type 2 (CR2), also known as CD21. The major histocompatibility complex (MHC) class II molecule is a cofactor for the infection of B-cells. Infection results in cellular activation and immortalization. The DNA genome of EBV encodes about 100 viral proteins and during viral replication, all these proteins are expressed [22].

After primary infection, the EBV genome becomes circular; forming episomes in B cells, and remains latent in these cells. During primary infection, as in infectious mononucleosis, the viral antigens expressed by peripheral blood B cells which characterized by the limited expression of a subset of viral gene products, including six nuclear antigens (EBNA-1, -2, -3A, -3B, -3C and -LP) and three integral membrane proteins (LMP-1, -2A and -2B),[23]. The nuclei of

the cells with latent infection contains a high copy number of EBV encoded RNAs (EBER-1 and EBER-2). In most asymptomatic carriers of EBV, the virus is occasionally replicated and infectious virions are then found in oral secretions [24].

1.5.3. Infection of epithelial cells

Epithelial cells generally do not express CD21, nor do they have HLA class II molecules on their surface. Therefore, entry of EBV into epithelial cells is evidently via a different method to B cells, although the exact mechanism remains unclear. Using recombinant EBV carrying a neomycin resistant gene, both CD21-dependent and -independent infection of epithelial cell lines has been shown in vitro [25]. Infection is reliant on gp85/25 complexes, which do not include the gp42 component essential for B cell infection [26]. Interaction between the viral BMRF2 protein and β1 or α5β1 on the basolateral surface of epithelial cells has also been suggested as an alternative CD21-independent mediator of viral entry [27]. In vitro, efficient cell to cell infection from EBV-infected Lymphoblastoid cell line (LCLs) to epithelial cell lines derived from a range of carcinomas has been described, as has transfer of virus from B cell membranes to epithelial cells [28]. This process requires cell to cell contact and is CD21-independent, although induction of CD21 expression enhanced infection efficiency [22]. Once the initial infection is achieved, it is possible that cell to cell spread amongst epithelial cells occurs [27].

1.6 Immune response to EBV

Epstein-Barr virus infection induced both cellular and humoral immune responses. During EBV primary infection, three antibodies (-IgG, -IgM and -IgA) are produced against EBV viral capsid antigen (VCA), two antibodies (-IgG and -IgA) are produced in response to early antigen D (EA-D), one antibody (-IgG) in response to early antigen R (EA-R) and three antibodies (-IgG, -IgM and -IgA) to membrane antigen (MA) [29]. Each of these antigens is a composite of several distinct viral proteins. EBNA is a complex of six distinct nuclear proteins, EBNA1, 2, 3A, 3B, 3C, and –LP, while antibodies to EBNA1 are most frequently detected by the conventional anti-EBNA assay. EA is a complex of immediate-early and early viral proteins (BZLF1, BALF2, BHRF1, BMRF1 and BMLF1 protein). Much of VCA reactivity is diffuse cytoplasmic fluorescence against virus-encoded nucleocapsid components (BcLF1, BFRF3, BLRF2, and the

glycoprotein gp110), which are expressed in late lytically infected cells. Most MA reactivity is directed against gp 350, the most abundant viral protein on the surface of lytically infected cells and on the viral envelope. The serum levels of anti-membrane antigen correlated with the nutralisation activity of the virus. Antibodies to VCA (IgA, IgG, and IgM) expressed lytically infected cells. These antibodies are almost always present in relatively high titres [30]. The serological reactivity against the two dominant EBNA proteins, EBNA1 and EBNA2, changes with time after EBV primary infection, Antibodies to EBNA2 appear before EBNA1 antibodies[30]. The VCA IgG antibodies are detected throughout life in healthy persons carrying EBV. IgM antibodies to VCA disappear within one to three months [31]. EBV neutralizing antibodies of IgM class appeared at the beginning of the infection and with low titer, while IgG is the predominant class with infection duration and with high titer.

Antibody responses to the latent membrane proteins (LMP1 or LMP2) have not been seen during infectious mononucleosis but these antibodies are only detectable in a small proportion of healthy carriers with the most sensitive assays [32]. Virus-neutralizing antibodies (anti-MA antibodies) are capable to prevent generalized spreading of the virus. However, the cellular immune response is more important than the humoral for restricting EBV infection. Natural killer cells and CD4+ and CD8+ cytotoxic T cells control EBV-infected B cells during primary infection [33]. In infectious mononucleosis, up to 40 percent of CD8+ T cells are targeted towards one replicative EBV protein sequence, whereas two percent are targeted to one latent EBV protein sequence [34]. After recovery, HLA-restricted cytotoxic T cells are important in controlling EBV latency, and CD8+ T cells are equally targeted towards replicative and latent antigens [35]EBV induces a strong cytotoxic T lymphocyte (CTL) response to multiple antigens. However, a great deal of the cytotoxic-T-cell response is targeted towards the EBNA-3 proteins [36].

EBV may escape immune surveillance in vivo in several ways [37]by altering the amino acid sequence within epitopes recognized by virus specific cytotoxic T lymphocytes (CTLs); via down-regulation of the peptide transporters, thus restricting the loading of MHC class I molecules with peptides derived from intracellular antigens [38], via down regulation of CTL immunodominant EBV proteins of

EBNA3A, EBNA3B, EBNA3C (EBNA-3, -4, and -6) as in some EBV-associated malignancies, such as Hodgkin's lymphoma [39]; via strong CTL response by certain dominant HLA alleles, such as B8, which mounts a strong response to an EBNA-3 epitope [40], or B27, which mounts a strong response to an EBNA-6 epitope [41] or A11, which mounts a strong response to an EBNA-4 epitope [42]. EBNA3B (EBNA-4) is a transformation-associated EBV nuclear antigen that has been shown to contain multiple HLAA11- restricted epitopes with different immunogenicity [37]. A high prevalence of EBNA3B (EBNA-4) mutations has been proposed as a mechanism of escaping the CTL response in certain HLA types [42].

Epidemiological studies have shown that mutation of the antigenically determined epitope of EBNA3B (EBNA-4) may play an important role in the development of EBV-associated malignancies [37]. EBV encodes several important proteins that show sequence and functional homology to diverse human proteins.EBNA-1 has been shown to block its own degradation [43] Since viral proteins are normally broken down by proteasomes to peptides for presentation to cytotoxic T cells, the ability of EBNA-1 to inhibit its degradation allows the protein to avoid triggering the activation of cytotoxic T cells [44]. Burkitt's lymphoma cells that are infected with EBV down-regulate the expression of several proteins which subsequently influence T cell cytotoxicity of the infected cells. These include the transporter proteins associated with antigen processing that convey viral peptides from the cytoplasm to the endoplasmic reticulum for antigen presentation, the cellular adhesion molecules that allow the cells to contact with each other and the MHC class 1 molecules that allow cytotoxic T cells to recognize virus-infected cells [45].

1.7 Oncogenic features of EBV

To be oncogenic, EBV must maintain its viral genome in the cell, avoid killing the cell, and prevent the cell from becoming a target for destruction by the immune system. Finally, the virus must activate cellular growth control pathways. Latent B lymphocytes infection established by EBV to maintain EBV DNA in cell. The EBV genome is maintained in these cells, either as multicopy circular episomes in the host cell or by integrating the viral DNA into the host genome [45]. The virus thus ensures transmission to cell progeny when B lymphocytes replicate. Activated phenotype in the infected B cells is

induced by Epstein-Barr virus latent genes... Although these cells are not transformed, if they proceed unchecked or acquire oncogenic mutations, they can become neoplastic [46].

In normal subjects, activated B lymphocytes expansion is prevented by the response of T-cell cytotoxicity against latent viral protein. Epstein-Barr virus eventually enters the resting B-cell memory compartment through normal differentiation of these cells. Only EBNA-1 is expressed in these cells. The EBV growth-promoting latent genes are not expressed, and so the cells are not pathogenic [47]. The limited repertoire of gene products also prevents frequent viral replication. Because cytotoxic responses to EBNA-1 are rare, EBNA-1-expressing lymphocytes escape immune surveillance. This then constitutes the viral reservoir. Some infected cells intermittently may enter the lytic cycle during which viral replication induced and subsequent cell death and virion release to infect other cells. [48]. with immune suppression, latently infected cells in the peripheral blood or persistently infected cells on the oropharynx increase in number [49]. The EBV activates intracellular signaling that play a role in the control of cell proliferation and mandated oncogenicity. This is achieved through diverse virally expressed genes that stimulate multiple intersecting cellular transduction pathways [50].

Nasopharyngeal carcinoma, Burkitt's lymphoma and Hodgkin's disease may emerge many years after the primary EBV infection. A clone of EBV-infected cells may initiate these tumors. The role of EBV in these late-onset malignancies is complicated. Because EBV is clonal, it clearly sets the stage for progression to frank tumor [51]. However, other factors may be important: specific failure of immune recognition; stimulation of B-cell proliferation by other infections; and/or appearance of secondary genetic aberrations or mutations [52,53].

1.8 Pathogenicity of EBV infection

1.8.1 Infectious mononucleosis.

Epstein Barr Virus can cause infectious mononucleosis, also known as "glandular fever". Infectious mononucleosis is caused when a person is first exposed to the virus during or after adolescence. Though once deemed "The Kissing Disease" recent research has shown that transmission of Mononucleosis not only occurs from

exchanging saliva, but also from contact with the airborne virus. It was predominantly found in the developing world [54].

1.8.2 EBV and Breast Cancer

The association between EBV and breast cancer is still quite controversial: EBV has been detected in subsets of breast cancer tumors [55], but negative results have also been obtained [56]. Low viral loads have been detected in breast cancer biopsy specimens, but the infected cells have not been clearly identified. Arbach and his colleagues [55] detected EBV DNA by quantitative PCR in whole tumors and micro dissected tumor cells. Half of tumor specimens contained EBV DNA with low copy numbers. In this study the viral load was highly variable from tumor to tumor and EBV genomes were heterogeneously distributed in morphologically identical tumors cells (there were some clusters of isolated tumor cells with relatively high genome numbers while other tumor cells from the same sample may be negative for EBV DNA). [55] detected EBNA-1 transcripts in almost all of the EBV-positive tumors and LMP-1 RNA in three of the 15 cases studied.

Hennard and colleagues [57] have reported that the 2B4 monoclonal antibody, which have been commonly used to demonstrate EBNA1 protein in tumor samples, cross-reacts with the MAGE4 protein. The MAGE4 protein is a cancer testis antigen which is expressed in a number of tumors types [57]. The authors recommend that this 2B4 monoclonal antibody should not be used to screen tissue samples for EBV [56,58]. The PCR studies of micro dissected tissues show that a small percentage of tumors carry EBV [59]. Interestingly, EBER expression has not been detected; it remains to be seen whether EBER-negative form of latency really exists [59] In conclusion, in those EBV positive cases, virus is present at low copy numbers and detectable only in a fraction of tumor cells [55, 59]. It is possible that although EBV does not have an etiologic role in the genesis of breast cancer, the virus might contribute to tumor progression [55,59].

1.9 Diagnosis of EBV infections

Serological diagnosis of EBV primary infection is classically based on detection of heterophile antibodies and on measurement of antibody reactivity for various EBV antigens, including viral capsid antigen (VCA) and members of the Epstein-Barr virus nuclear antigen

(EBNA) family Characteristic for EBV primary infection are positive VCA IgM and IgG results and lack of EBNA-1 antibodies [29]. The EBNA-1 test becomes positive; VCA IgM antibodies usually disappear in convalescence, while VCA IgG persists for life. However, even in immunocompetent seropositive individuals EBNA-1 antibodies can sometimes remain negative, and more often in immunocompromised patients or in chronic mononucleosis [60]. A sign of viral reactivation can be the reappearance of VCA IgM antibodies, but VCA IgM in some patients remains detectable long after EBV infection even with no known reason [61]. To date, no single commonly accepted serological criterion exists for EBV reactivation, decrease of EBNA IgG [62], increase of VCA IgG [63] or simultaneous positivity EBNA IgG [64]. In Finland, Lehtinen and colleagues [65] have reported that elevated EBV EA and EBNA antibody levels were associated with a statistically significant excess risk of malignant lymphoma/leukemia and breast cancer. These elevated antibody responses may be due either to destruction of neoplastic EBV positive B-cells and/or to activation of latent EBV infection early in the lymphoma genesis.

Measurement of IgG avidity has been shown to be a powerful tool for differentiation of primary and secondary infections of various pathogens [66]. Also in EBV serodiagnosis, avidity of VCA IgG separates primary and secondary infections both in immunocompetent and immunocompromised individuals [67]. In recent years, more economical and objective tests for antibodies against recombinant EBV proteins have been introduced using enzyme-linked immunosorbent assay technology and related methods [68].

Some difficulties have been encountered in detecting EBV at the tissue level, which is necessary for tumor diagnosis. The low copy number of viral genomes and the restricted pattern of viral antigen expression limit the reliability of many standard techniques including Southern blot hybridization, antigen detection and in situ hybridization (ISH) of mRNA or DNA targets [69]. EBV can be detected in normal tissue using PCR. Monoclonal antibodies to the EBV latent proteins EBNA-2 and LMP-1 have been used to demonstrate viral expression in lymph proliferative disease, Hodgkin's disease and nasopharyngeal carcinoma [70]. Due to variable expression of EBV latent proteins and technical difficulties

with fixed tissues, the failure to detect these proteins does not necessarily indicate the absence of EBV [69]. New PCR-based methods show promise in permitting fast quantitation of viral DNA in fluidic or cellular samples [71].

2. Human Cytomegalovirus

2.1. Characteristic features of human cytomegalovirus (HCMV)

Human cytomegalovirus is a virus with common infection in human [72-74]. It is highly species-specific and infects different cell types. HCMV belongs to the subfamily of herpes viruses. The virion of HCMV has a typical herpes virus structure. It consists of an inner core of a double-stranded linear DNA molecule surrounded by a nucleocapsid and a thick layer of tegument protein that is surrounded by a lipid bilayer envelope [75].

2.2. The HCMV genome

HCMV is the largest and most complex of all known herpes viruses. It consists of a genome of approximately 235 kbp and containing 252 open reading frames (ORFs), which was believed to encode 180 proteins. However a recent study suggests that this number may in fact exceed 750 proteins [76], revealing that HCMV may be far more complex than previously believed. Interestingly, only about 50 proteins are believed to be essential for its replication and the vast majority of HCMV proteins interfere with cellular and immunological functions to enable the virus to coexist with its host [77].

Herpes virus genomes are not simple lengths of unique DNA, but characteristically contain direct and inverted repeats. The reasons for this are not known, but it is intriguing that similar structures appear to have arisen independently on several occasions during herpes virus evolution [78].

2.3. Human cytomegalovirus structure and life cycle

HCMV has the prototypical herpes virus virion structure and the replication cycle has a well-controlled cascade of gene expression .The virus has an icosahedral protein capsid that contains the 235kbp double stranded DNA. The capsid is surrounded by a proteinaceous tegument and an outer lipid envelope [79]. Through membrane fusion sequences that involve viral lipid envelop glycoproteins and cell outer membrane CMV enter the cell. Once the fusion of these two membranes occurs, the DNA containing protein capsid and the

tegument proteins are released into the cell [80]. During the lytic infection, viral immediate-early genes are expressed [81]. Stimulation of viral early genes and modulation of the host cell environment is achieved by the production of viral immediate-early proteins as a response to expression of viral immediate-early genes. The replication of double-stranded viral genomic DNA influenced by viral immediate-early genes protein production. Following DNA replication, the immediate-early genes initiate the expression of late viral genes.

The viral late proteins are mainly structural components of the virion that assist in the assembly and egress of newly formed viral particles [79]. Immediate-early genes in HCMV can be silenced in certain cell types upon infection though, which results in a latent infection [82]. A latent infection is characterized by the minimization of viral gene expression and the inhibition of the assembly and egress of new viral progeny [80]. Latent infections can reactivate into a lytic infection upon certain environmental cues, which causes disease and allows viral spread [83].

2.4. Immune response

A critical component in malignancy, specifically in inflammation-associated malignancies, is the loss of normal anti-tumors immune functions in the tumors microenvironment (TME). Epidemiological data increasingly supports the existence of this concept after finding striking increases of certain cancers specifically infection-related cancers in immunocompromised individuals [84]. During the last decade, an increased understanding of the molecular mechanisms responsible for mounting a proper anti-tumor immune response show that both the protective capacity of the immune system against tumor cells (host related) and the evasion of tumor cells from attack by immune cells (tumor related) can lead to a failure to mount a proper anti-tumor- immune response. There are several known key factors interfering with this process, such as T cell energy, the existence of regulatory T cells and systemic defects of dendritic cells DCs derived from cancer patients. Furthermore, tumor-related factors, including secretion of immunosuppressive cytokines, resistance to apoptosis and deficient expression of immunomodulatory molecules and MHC-I antigens play an important role in modulating the immune response to cancer [85].

Cancer patients who have tumors that are heavily infiltrated with CTL and NK cells, have a better prognosis in terms of disease free and overall survival time at all stages of clinical disease than patients who lack such abundant killer lymphocytes [86]. In contrast, presence of immunosuppressive cells including T regulatory (Tregs) that can suppress the action of cytotoxic lymphocyte function and consequently associates with poor patient outcome [87]. In addition to immunosuppressive cells, the secretion of immunosuppressive cytokines such as IL-10, transforming growth factor beta (TGF-β) by cells in the tumor microenvironment also play a major role in inhibiting the antitumor immune response [88]. These molecules can block DC maturation; attract regulatory T cells to the tumor microenvironment, which subsequently leads to further inhibition of cellular anti-tumor immune response [88]. Moreover, HCMV gene products produced by infected tumor cells could dramatically alter the host's ability to recognize tumor cells. Through many years of co-evolution with the host, this virus has developed several immune evasion strategies to allow persistent infection and viral spread without harming its host.

The ability to evade from recognition by the immune system is necessary for the survival of cancer cells as well [89]. HCMV keeps a balance with its host's immune system, it stimulates the immune response and induces the inflammation, but in parallel, it escapes immune recognition through multiple sophisticated pathways.MHC peptide presentation is required for CD8+ cytotoxic tumor killing and suppression of antigen presentation will prevent T cell mediated killing of HCMV infected cells. In addition, HCMV US2 and US3 have been shown to decrease surface expression of MHC-II thereby evading recognition by CD4+ T cells [90]. In tumor tissues, it has been shown that the HCMV protein pp65, which is consistently detected in human glioma, and it is known to phosphorylate HCMV IE-72 peptides derived from the IE-72 protein. Subsequently, pp65 blocks IE-72 presentation as target epitope preventing it for being recognized by immune system [91], which may cause HCMV mediated immune evasion of infected tumor cells.

Natural killer cells provide an important line of defense in killing tumor cells and viral infected cells. HCMV subverts NK mediated killing of infected tumor cells by several different strategies.

It has evolved a unique strategy to evade its own virally mediated down regulation of MHC-I antigen to overcome NK cell recognition of infected cells [90]. An HCMV-encoded MHC-I homologue UL18, inhibits NK responses by triggering LIR-1 [92]. HCMV is known to counteract the effects of HCMV specific antibodies through two HCMV encoded Fc gamma receptor homologue, gpUL119-118 and gpTRL11, which binds to HCMV infected cells, covering antigens presented on the cell surface [93]. Furthermore, the complement system provides a main line of immunologic defense against invading pathogens, to prevent activation of this pathway. HCMV interferes with complement activation through induction of the cellular proteins CD35, CD46 and CD55 that will inhibit opsonisation by phagocytic cells and subsequently prevent complement mediated cell lysis [94].

2.5.Promotion of tumor genesis by Human Cytomegalovirus (HCMV) in breast cancer

The prevalence of HCMV ranges from 60–80% in developed countries to approximately 100% in developing countries [95]. After a primary infection, HCMV establish latency in a small percentage of peripheral blood monocytes [96] and in CD34+ myeloid progenitor cells in bone marrow [97]. The virus can be reactivated by inflammation and immune activation and may lead to severe disease in immunosuppressed patients [98]. However, transcripts associated with HCMV latency and expression of immunogenic proteins (e.g., latency unique natural antigen) has been reported during latency and may stimulate the immune system [99]. In about 90% of HCMV-seropositive mothers, reactivated HCMV in breast milk can cause subclinical infections in infants; as a result, HCMV is found in 30–40% of 1-year-old children [100]. These observations reveal an additional site of persistent HCMV infection that may have implications for breast cancer development. HCMV infection contributes to the pathogenesis of chronic inflammatory conditions such as inflammatory bowel diseases [101], cardiovascular diseases [102]. In fact, the inflammatory milieu is an excellent environment for reactivation of latent HCMV. Reactivated HCMV can then boost inflammation by inducing expression of COX-2 and 5-lipoxygenase and by enhancing the production of inflammatory factors such as leukotriene, prostaglandins, interleukin (IL)-1, IL-6, IL-8, IL-10, monocyte chemoattractant protein 1, and macrophage inflammatory

protein 1 [102]. In chronic inflammatory diseases, the constant release of inflammatory cytokines may result in migration of more latently infected peripheral blood monocytes to inflamed tissues, which can differentiate into macrophages (MQ)/dendritic cells (DCs) and reactivation of latent virus[101]. HCMV infection can then spread to nearby cells of various types, including vessel endothelial, smooth muscle, epithelial, glial, stromal, and neuronal cells, fibroblasts, and hepatocytes and may exacerbate malignant disease by enhancing the production of inflammatory factors. Chronic inflammation has long been associated with cancer and is now an established hallmark [103], along with genetic modifications, lack of response to anti-proliferative signals, self-sufficient and uncontrolled cellular growth, evasion of apoptosis, activated oncogenes, and dysfunctional tumor suppressors, all of which lead to oncogenesis. CMV acute infections may be followed by remission or induction of chronic or latent infection and subsequently lead to cancer development through free radicals production, which contribute to nitration of DNA basis, oxidative damage, and increased risk of DNA mutations [104].

HCMV infection has been linked to breast cancer in several studies over the last decade. Studies that relied on HCMV serology showed increased levels of IgG antibodies against HCMV in breast cancer patients [33,105]. In particular, the higher prevalence of HCMV in hypothesis HCMV infection contributes to breast cancer. Previous studies have shown a very high prevalence of HCMV proteins and nucleic acids in tumor tissues and in lymph node and brain metastases of women with breast cancer [106]. More than 90% of primary tumors, 94% of lymph node metastases, and 98% of brain metastases of breast cancer are HCMV positive. Interestingly the infection is largely restricted to tumor cells, although some endothelial cells and inflammatory cells may also be positive [107].

Although HCMV is highly prevalent in certain tumor tissues, its potential causative role in tumor development is poorly understood. Since HCMV cannot cause malignant transformation of cells, it is not considered to be oncogenic [108]. During evolution HCMV has co-exist in human and has developed mechanisms to avoid viral elimination by the immune system, establishes latency and adapted to persist in the immunocompetent host. HCMV infection may protect tumor cells from apoptosis by expression of HCMV-IE anti-apoptotic

proteins IE1, IE2, UL36, UL37, and UL38 to avoid immune clearance by NK and cytotoxic T cells [109].

HCMV-UL36 expression in tumor cells may contribute to tumor genesis of the cells by expression of a viral cell-death suppressor (vICA) [110]. In addition, HCMV infection in tumor cells may enhance tumorgenecity of these cells by increasing AKt activity (cellular protein kinase B) and alteration in cellular metabolism and inhibition of apoptosis [111]. CMV is with regulatory oncogenic effects on tumor cells which lead to transformation in to more aggressive phenotype and poor response to immunotherapy and chemotherapy. Thus, like oncogenic DNA viruses such as HPV and adenovirus, HCMV can increase the malignancy of tumor cells by modulating regulatory and signaling pathways to enhance their proliferation, survival, invasion, motility, and adhesion and by providing sophisticated strategies to avoid immune recognition and killing of infected cells [111]. HCMV infection induces cell cycle arrest by inhibiting cyclin-An expression, resulting in inhibition of cellular DNA synthesis and enhance cell cycle progression by increasing cyclin-E and it is associated kinase activity (CDK2) [112]. Furthermore, HCMV infection can drive neoplastic transformation in vulnerable adult stem cells by causing chromosomal damage in some positions [113]. Interestingly, the possible targets are potential tumor suppressor genes, whose deletion has been linked to glioma and primary breast cancer, respectively [114]. HCMV-UL76 protein can induce DNA damage, apoptosis, cell cycle arrest and inhibition of DNA repair machinery [115]. In breast cancer patients, we found a high prevalence of HCMV infection in lymph node and brain metastases [106,107]. The main cause of death in these women is metastases to distant organs, which occurs within 3 years after diagnosis in about 10–15% of patients [116].

HCMV infection may influence breast cancer metastasis by different means, possibly by increasing expression of factors important in inflammation and angiogenesis and by inducing epithelial-to mesenchymal transition [117]. HCMV infection enhances the production of potential inflammatory factors, including prostaglandin E2 and leukotriene B4, by inducing expression of COX-2 and 5- lipoxygenase, respectively. These inflammatory factors are also involved in cellular proliferation and migration, angiogenesis, and

metastasis formation and are targets for nonsteroidal anti-inflammatory drugs (NSAIDs) [118]. Interestingly, NSAIDs also have anti-HCMV activity. The hypothesis of that inflammation is a risk factor for tumor development is supported by the finding of epidemiological studies. Long-term use of NSAIDs is strongly associated with a decreased incidence of breast cancer and other malignancies [119]. Vascular endothelial growth factor (VEGF), which is important in vascular permeability and tumor angiogenesis, is highly expressed in breast cancer metastases [120].

2.6. Diagnosis of HCMV infection

HCMV infection cannot be reliably distinguished based on clinical grounds from other infectious agents causing similar illnesses, such as EBV and hepatitis virus. A number of laboratory investigations are used to diagnose HCMV infection, which include:

2.6.1. Serology

Serologic tests for detection of HCMV specific antibodies are useful for determining whether a patient had HCMV infection or not, and to determine whether the infection occurred recently by detecting the conversion of HCMV-IgG antibodies from negative to positive, or by demonstration of HCMV-specific IgM antibodies. However, HCMV-IgM lacks specificity for primary infection, due to possible false positive results, and should be followed by additional serum tests over time [79]. Acute HCMV infection can be also confirmed by performing an avidity test for HCMV-IgG antibody, which increases with time after initial infection. Demonstration of low HCMV-IgG avidity can improve the accuracy of identification of recent infection [121]. Furthermore, a neutralisation assay can be used as a reliable method for differentiating between acute primary and non-primary infection [122].

2.6.2. PCR

PCR is a method for detection of HCMV infection used at most clinical laboratories today. It gained its popularity because it is rapid, sensitive, specific, provides a quantitative read out and it is amenable for automatic sample processing [123].

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