The Role of Human Cytomegalovirus Infection in Glioblastoma Multiforme Pathogenesis: Review of the Literature

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Abstract

Gliomas are the most common brain tumors and include a variety of histologic types and grades of malignancy. They arise from glial cells and represent approximately 70% of the primary brain tumors. Glioblastoma, also known as diffuse astrocytoma, WHO grade IV or glioblastoma multiforme (GBM), is the most common and fatal primary malignant brain tumor in adults. Previous researches have shown the presence of Human Cytomegalovirus (HCMV) nucleic acids and Proteins in GBM tumor tissue, suggesting that the virus may implicate in its pathogenesis, by affecting tumor stem cell factors, angiogenesis and immune pathways. However, this association remains controversial as many other studies could not detect HCMV in those tumor samples.

Moreover, other investigators have had variable success in detecting HCMV infection in tumor cells, and the common consent is that very low levels of viral proteins and nucleic acids could be identified. Although HCMV has not been considered to be oncogenic, a possible oncomodulatory role has been suggested. Virus infection, especially by DNA viruses and retroviruses, which may cause insertion of viral DNA sequences into the host genome, a situation which often activates the host defense systems, could lead to GBM development. In the current review, is presented the recent aspects regarding the possible association between HCMV infection and GBM development. Specifically, the outcomes of previous and recent studies, both positive and negative, will be presented, and the potential mechanisms through which HCMV infection may contribute to GBM development by affecting tumor cell initiation, progression and metastasis formation, and the potential theories to explain discrepancies reported in the literature will be suggested.

Keywords: Cytomegalovirus, Oncovirus, Brain tumor, Glioblastoma Multiforme.
Introduction

Gliomas are the most common and aggressive primary brain tumors that cannot be completely resected surgically. Among them, malignant glioma is associated with high recurrence and high mortality rates. Glioblastoma, also known as diffuse astrocytoma, WHO grade IV or glioblastoma multiforme (GBM), is the most frequent adult primary malignant brain tumor, shows a great morphological and genetically heterogeneity, its frequency varies between 12%-15% of all intracranial tumors, and 50% to 60% of astrocytic tumors(1,2).

GBM is divided into two types, primary and secondary. A rate of 90% of GBM cases develop de novo, as primary glioblastoma, from normal glial cells by multistep tumorigenesis and grows within 3 months, whereas the remaining 10% are secondary neoplasms, developing through Progression from low-grade tumors, diffuse or anaplastic astrocytomas or oligodendrogliomas, which take about 4-5 years. Secondary GBM is caused by a malignant transformation from a ower grade brain tumor, as mentioned, and/or with mutation in the gene of isocitrate dehydroge-genase (IDH). GBM is classified to the following histopathological types Classical, Proneural, Neural and Mesenchymal based on the gene expression profile, grows more slowly and shows better prognosis and is diagnosed mostly at a mean age of 39 years(3-5).

GBM patients have severe clinical signs and symptoms, 10% of them survive within three years, only 3-5% survives for more than 5 years, whereas the median survival after the final diagnosis has been observed to be 12 to 15 months(1,6). Its poor prognosis could be attributed to its extremely invasive and proliferative nature as is characterized by a highly abnormal vascularization, displays resistant to the common chemotherapy and radiotherapy and in general is difficult to be completely removed surgically despite the variety of modern therapies(7).

GBM is a rare tumor with global incidence of less than 10/100,000 people, 5.26/100,000 population or 17,000 new cases/year in the USA, and 3.19/100,000 adults each year in Europe and America(8,9).

GBM’s etiology has not been fully elucidated and still remains unclear. Genetic influences in combination with environmental risk factors have been suggested as pathogenic factors of GBM which are involved in the initiation of glioma and its progression(2,10). Its familial form has been described for 1% of case, however, the genetic background for development of GBM familia reform is different from those arising spontaneously, whereas it has also been suggested that is a spontaneous tumor(10-12).

GBM development has been associated with genetic diseases such as Li Fraumeni syndrome, and Neurofibromatosis type I (NF1), Multiple Endocrine Neoplasia type IIA (MEN IIA), Turcot syndrome, and tuberous sclerosis(13-16). Individual’s height and body mass index (BMI) have been associated with its development as high values of those two variables increase the risk of GBM incidence(17). Moreover, an hypothesis on the implication of gender hormones in GBM development was suggested based on the observation that a higher risk of GBM was identified in postmenopausal females(18). Acquired head injuries, as a result of a brain contusion, may predispose to the initiation of GBM(19). An increased risk has been observed following exposure to ionizing radiation which is thought to induce DNA breakage and cell cycle abnormalities across time (2) or chemical agents(20).
Epidemiologic retro- and prospective studies have shown that viruses are the second most important risk factor for cancer development in humans(21). Various cancer types have a viral etiology and some viruses have been identified as cancer causing factors. Significant associations between hepatitis B and C viruses (HBV/HCV) and hepatocellular carcinoma have been found and certain types of human papillomavirus (HPV) have been indicated to cause cervical cancer, whereas possible oncogenesis has been suggested after specific tissue bacterial/viral infections, such as Epstein-Barr virus (EBV) in Burkitt lymphoma, and H. Pylori-associated gastric cancer(22-25).

Viruses may involve in oncogenesis and tumor development by inducing immunosuppression, modifying host cells through inducing oncoproteins, or altering the host cell proteins expression at viral integration sites(26,27).

More recently, increasing emphasis has been focused on a viral etiology of gliomas as they might serve as oncomodulators(26,28). Oncomodulation is the ability of viral proteins and non-coding RNAs to promote oncogenic processes without direct onco-transformation, but through disruptions in various intracellular signaling pathways(28). Several viral pathogens have been suggested to implicate in GBM pathogenesis. Mice injected with JC virus, the human polyomavirus most associated with progressive multifocal leukoencephalopathy, have been referred to develop malignant gliomas, and SV40 has been identified in human GBMs(29,30). In addition, hamsters injected with BK virus, another human polyomavirus, have been found to develop ependymomas(31). Recent reports, have involved Human Cytomegalovirus (HCMV, or human herpes virus-5/HHV-5) in high-grade gliomas pathogenesis, an hypothesis that has led to controversial outcomes(32,33).

Although elevated expressions of HCMV components in glioma tissue have been reported, it still remains unknown the possible role of HCMV infection in the initiation and progression of glioma, despite the fact that information on possible oncomodulatory functions for various viral proteins have reported (34-37). However, conflict results have also been reported. In some researches, no HCMV genes or proteins were identified in glioma samples(38,39). Those controversial results lead to an uncertain role of HCMV in the pathogenesis of glioma and the involvement of viral factors in GBM etiology(40).

Moreover, conflicting reports have been focused on the ability to detect HCMV proteins and nucleic acids in GBMs samples, although there is a general agreement that viral activity is well below the limits of standard diagnostic tests. The aim of the current review was to present recent aspects regarding the possible association between HCMV infection and GBM and the potential mechanisms through which HCMV infection may contribute to GBM pathogenesis by affecting tumor cell initiation, progression and metastasis formation, and the potential theories to explain discrepancies reported in the literature will be suggested.

**HCMV infection**

HCMV is a 235-kb double-strand DNA virus that encodes more than 200 viral proteins including immediate-early and pp65 proteins and at least 14 microRNAs, and non-coding transcripts(41-43).

HCMV is prevalent in up to 70%-90% of the general population, and it persists for life long after initial infection, whereas other studies report that approximately 50% to 90% or 100% of adults have been
infected, as indicated by the presence of serum IgG antibodies, especially in cases of periodic reactivations(44).

Cells with productive (lytic) infection are large and contain single basophilic nuclear inclusions with a clear zone or halo(45). HCM virus consists a neurotropic virus, which can infect brain microvascular endothelial cells, microglial cells, pericytes, neurons, astrocytes, neural stem cells and neural precursor cells, and impact neuronal differentiation in fetuses. It can also go through brain endothelium by virus-infected leukocytes(46).

Initial infection in the immunocompetent host is typically asymptomatic or mild, although a mononucleosis-like syndrome can occur. Its reactivation or infection in immunosuppressed individuals can lead to various infections including encephalitis, hepatitis, pneumonia, colitis, and retinitis and can be life-threatening and even fatal complications, especially in individuals with AIDS or cancer(47). After a primary infection in the immunocompetent host, it establishes latency and persistence in myeloid origin cells, and periodic asymptomatic reactivations are considered to occur during life without clinical signs or symptoms of infection(48).

Patients with previous HCMV infections are at higher risk of developing acute and chronic rejection, bacterial and fungal infections, cardiovascular diseases, post-transplant diabetes and some malignancies in the post-transplant period(49-51). Those conditions could be attributed to indirect effects of HCMV infection (51), as the virus is difficult to be detected in affected organs at the time of diagnosis(52). However, recently, the use of sensitive techniques for detection of HCMV, such as the Real-Time Quantitative Polymerase Chain Reaction (qPCR) revealed that the presence of HCMV in kidney grafts was associated with decreased organ function and graft survival(53), suggesting that HCMV causes direct, in addition to indirect effects, in the graft. Congenital infection by HCMV is the most common cause of severe neurological damages including optic atrophy, hearing loss, and microcephaly in many countries, worldwide(45).

### HCMV Infection and Oncogenesis

HCMV is not a typical oncogenic virus; however its proteins are implicated in tumor promotion signalling pathways. It encodes more than 200 viral proteins, of which only about 45 have been identified to be involved in virus replication(41,54). Stern-Ginossar et al. showed that 751 unique HCMV proteins are translated in infected cells based on ribosomal profiling, which means that this virus is more complicated than previously considered, and that the majority of HCMV proteins must involve in other functions during the virus life cycle than implicating in replication and formation of new virus particles(55).

Various HCMV encode proteins can under certain occasions initiate cellular transformation or through other pathways contribute to tumour development and provide mechanisms that are associated with the hallmarks of cancer(56). The possible role of HCMV in cancer development was firstly reported in a study by Geder et al., who observed a frequent presence of HCMV in prostate cancer, and isolated a virus strain from tumors that was oncogenic in vitro and in immunodeficient mice(57). More recent reports did not confirm that HCMV was able to transform normal human cells, and it was considered that the virus was not oncogenic. A virus is considered as an oncogenic in case it encodes gene products that can induce
cellular transformation under certain circumstances, such as HBV, EBV, HPV, SV40, and adenoviruses. The possible indirect influence of HCMV on tumorigenesis has been contributed to its ability to promote an oncogenic process characterized by disruptions in intracellular signalling pathways, transcription factors and tumor suppressor proteins in an appropriate genetic environment provided by tumor cells(58). This process is called oncomodulation.

HCMV proteins are involved in the cell cycle control, inhibits apoptosis, induce telomerase activity, cellular migration and angiogenesis and therefore provide oncomodulatory mechanisms(59-61). Moreover, induce oncogenes expression, regulate tumor suppressor genes expression, induce p53 mutations and specific chromosomal breaks, inhibit DNA repair pathways, regulate epigenetic functions and cellular proliferation(62,63), and provide immune evasion functions(64). HCMV was also found to cause DNA damage. Its protein UL76 was revealed to induce chromosomal abnormalities(65), and the virus has been associated with a specific damage at several chromosome loci 1, 1q21, 1q23 and 1q42 (66,67).

HCMV protein expression in mucoepidermoid cancer is also associated with activation of known oncogenic pathways such as EGFR, ERK and amphiregulin(68).

Experimental data suggest a direct molecular association between the expression of US28 protein and tumorigenesis. In addition, US28 has also been shown to activate the transcription nuclear factor B (NF-kβ), a critical regulator of immunity, stress responses, apoptosis, cellular differentiation and migration(69). The US28-encoded protein (pUS28) is one of four G protein-coupled receptors (GPCRs) encoded by the HCMV genome and is a potent signaling protein in the context of lytic infection(70,71). This protein is a chemokine receptor that promotes angiogenesis and tumor development through the activation of NFkB induces cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) expression, and mediates PGE-2 and leukotrienes production that are both effective inflammatory mediators(72,73). COX-2 and PGE-2 are over-expressed in a number of different cancers and increased COX-2 expression is often associated with poor prognosis(74). HCMV induced COX-2 expression, and activation of Ras/Erk and PI3K/AKT signalling pathways may further induce and maintain a paracrine loop leading to possible cellular invasion into surrounding stroma(75). PGE-2 also induces cellular proliferation, angiogenesis, inhibition of apoptosis and stimulation of invasion, and can contribute to the production of a tumour promoting inflammatory microenvironment. It has also been identified an association between HCMV protein expression and COX-2 expression in medulloblastoma, suggesting that HCMV may regulate COX-2 expression in those tumors(76). The US28 protein, has also several characteristics resembling a viral oncoprotein(72,77,78). Its expression in NIH3T3 cells makes them tumorigenic in nude mice(73,78), which involves VEGF production and COX-2 expression(78). Moreover, its expression in transgenic mice leads to intestinal adenomas and adenocarcinomas(77), by inhibiting glycogen-synthase 3β (GSK-3β) activity, resulting in an accumulation of β-catenin and increased expression of Wnt target genes which are implicated in the cell proliferation control(77).

Experimental observations also suggest that HCMV can be oncogenic. A HCMV gene locus, the transforming region II (mtr II), a 980-bp sequence, was first found to transform fibroblasts in experimental animals(79,80).
Cellular transformation has been identified in case of expression of the HCMV proteins IE72 or IE86 together with the adenovirus E1A protein through a hit-and-run mechanism(81). HCMV infected epithelial cells can also activate extracellular latent transforming growth factor beta 1 (TGFβ1) through induction of metalloproteinase 2 (MMP-2), which is mediated by the HCMV proteins IE72 or IE86(82). Induced MMP-2 activity could mediate the degradation of the extra cellular matrix(83), which would contribute to the formation of a metastasis. The HCMV IE proteins can bind to p53, Rb and degrade p 21, and thereby modulate cell cycle regulation, induce telomerase activity(61) and downregulate tumor suppressor proteins, which may contribute to oncogenic transformation(60).

Epithelial cells can undergo a transition into mesenchymal cells, known as epithelial to mesenchymal transition (EMT), which results in the loss of cell-to-cell contacts and essential remodelling of the cytoskeleton. EMT is implicated in normal physiological development, however, EMT and mesenchymal to epithelial transition (MET) in tumor pathology, are involved in meta-static capacity of epithelial tumors. TGFβ1 regulates the EMT process. HCMV induces TGFβ1 production, thus HCMV contributes to the EMT process(84). HCMV infected and non-infected epithelial cells treated with TGFβ in vitro, were shown to undergo morphologic and transcriptional changes similar of EMT(82). HCMV US28 can interfere with the activity of expression of GSK3β, which phosphorylates and regulates the stability of oncogenic transcription factors such as the Smads and Snail that can trigger an EMT process(75).

HCMV infection leads to the production and release of numerous cytokines some of which may adversely affect cell survival(85). The HCMV interleukin 10 homolog (cmv IL10) has been shown to mediate local tumor immunosuppression and activation of STAT3, a transcription factor that can promote oncogenesis by being constitutively active through various pathways(86).

The specific role of HCMV infection in Glioblastoma Multiform Pathogenesis

The association of infectious pathogens with neoplasms, such as viruses and bacteria, has been considered recently(87). The most common and prevalent DNA viruses that are associated with cancer development are hepatitis B and C virus (HBV/HCV), human adult T-cell leukemia virus type 1 (HTLV-1), Epstein-Barr virus (EBV), Kaposi sarcoma-associated herpes virus (KSHV), human papillomaviruses (HPV)16 and 18, and Merkel cell polyomavirus(28). Polyomavirus and herpes viruses have been detected in various frequencies in primary malignancies of the centralnervous system (CNS). Other associations have involved herpes viruses, such as herpes simplex virus 1 and 2 (HSV-1, 2/HHV-1, 2), varicella zoster virus (VZV/HHV-3), EBV (HHV-4), human cytomegalovirus (HCMV/HHV-5), and human herpes viruses 6, 7, and 8 (HHV-6,7,8)(88). However, previous reports have been resulted in contradictory outcomes regarding the link between chronic viral infection and primary CNS malignancy(88,27).

Several viruses have been linked to the etiology of brain tumors including HCMV (27,89). In the case of brain tumors, there is contradictory and/or controversial evidence linking many of those viruses, especially HCMV(87,90). HCMV is highly prevalent in human populations, and following resolution of primary infection, persists for the lifetime of its host in a latent state, periodically reactivating during periods of stress and immunesuppressions(91).
Because of its relationship for glial cells and its ability to reduce apoptosis, increase cell invasion, activate telomerase, and enhance angiogenesis in tumor cells(61,92), a large amount of surveys have investigated the role of HCMV in glioma etiology.

It has been shown that certain common viruses such as HSV-1, HSV-2, EBV, HCMV are chronically present in the sera of patients with GBM, but not necessarily in their tissues. It is possible that those virus groups are associated with glioma progression, as it was detected viruses in GBM, but not in lower grades of glioma(89).

HCMV has been detected in many tumor types(76,93-101), and the relationship between the virus and cancer has been investigated most extensively in GBM(32,102-104). The reported pre-valence of HCMV in GBMs is ranged from 0% to 100%(38,39,103,105-118). Clinical observations support the relation of HCMV in GBM pathogenesis(118-123), although the exact mechanism is not well understood. Cobbs et al.(32) first described the potential association between HCMV infection and malignant glioma, which is considered to modulate the malignant GBM phenotype(124). A consensus statement by Dziurzynski et al. summarized the data for HCMV expression in gliomas due to the variability in techniques for HCMV detection in gliomas and demonstrated that HCMV was expressed in most human glioma samples with sensitive assays(124).

HCMV DNA and protein products have been identified in multiple tumor types(96,102,103,125,126) including low and higher grade glioma such as GBM samples(32), and in regards to GBM proteins and nucleic acids, have been identified in up to 90% of its specimen(32,94,127-129) and in some other cancers(95,130). Cobbs et al.(32) reported that HCMV gene products and nucleic acids were present in all 27 glioma samples investigated, as assessed by immunohistochemistry (IHC) and in situ hybridization (ISH) techniques, without being detected in other brain tissue. Similar studies confirmed that observation(106,107,131,132), while Mitchell et al. (106) found that a large rate of GBM samples were positive for HCMV immediate-early (IE)1, pp65 protein or glycoprotein B using IHC, ISH and PCR techniques. Scheurer et al.(107) found that the majority of glioma samples had HCMV viral genes and that the number of HCMV positive cells was higher in GBM compared to low grade gliomas, observation that was confirmed by similar studies(94,110). A recent study based on IHC technique was found pp65 positivity in gliomas, medulloblastomas, CNS lymphoma and meningiomas(133), whereas it has also been recorded that 25% of GBM patients had detectable levels of a HCMV micro RNA (miRUL112-3p) in their blood(134). In another study which was based on PCR techniques were detected 20 different regions of the HCMV genome and was also found that HCMV positivity was much more likely in GBM samples compared to epilepsy samples(135).

Stangherlin et al. emphasized that few malignant cells seem to contain viral DNA, transcripts and/or proteins indicating that HCMV infection or replication is not equally effective in all malignant cells(102). The virus has been detected in a high percentage of malignant glioma cells in vivo, but not in adjacent normal brain cells(32,106,107,136-138). In the HCMV genome have been identified 173 open reading frames (ORFs), however in GBMs samples have been detected only a few amount of its gene products such as US28, IE1, pp71, and glycoprotein B(139). Viral DNA, RNA and proteins were HCMV-positive in the majority of tumor cells in human GBM, including both anaplastic and low grade gliomas, but those studies have used different and non-similar methodological procedures, thus, this absolute of standardization consists a crucial problem(32,107).
On the other side, controversial findings have been reported regarding the presence of HCMV in glioma genomes, and more recent studies have also provided controversial outcomes regarding the relationship between HCMV infection and gliomagenesis(38,39,69,114-116,140-143).

Solomon et al. in a meta-analysis of 16 studies reported that there was no clear evidence for a possible role of HCMV infection in GBM tumorigenesis(37). Similarly, specific roles of other herpes viruses in glioma initiation have not been demonstrated to date(88,137,144-147). Baumgarten et al. did not confirm the observations by Cobbs et al., whereas Cobbs et al. stated that Baumgarten et al. did not use the carefully optimized protocol established in his lab, which is crucial to detect low level HCMV infection in GBM samples(115,140,148).

Recent conflicting reports showed no association of HCMV in brain tissue(90,149), whereas the roles of HCMV in glioma development and progression were also controversial(118). Similar studies recorded no detectable HCMV protein or genomic material in glioma samples (39,105), and a recent report using PCR techniques has also failed to detect HCMV glycoprotein B and IE proteins in GBM patient peripheral blood or tumor samples(143). Moreover, an analysis of glioma samples using IHC and PCR techniques found no HCMV viral products in the samples(150).

The main reasons for the mentioned controversial observations were the variability of HCMV detection depending on assay sensitivity(143), the variations in the HCMV seropositivity among different ethnic populations(116,151), and the use of different cohorts. In regards to the first reason, differences in protocols and experimental conditions used or traditional detection techniques, such as PCR, ISH, and IHC assays, could lead to differences in sensitivities for detecting low levels of viral gene expression. Although differences in HCMV seropositivity have been investigated, there is currently no clear association between HCMV seropositivity and incidence of GBM. HCMV seroprevalance is lower in Whites than in Blacks and Hispanics, however GBM incidence is higher. Moreover, HCMV seroprevalance is higher in females than males, whereas GBM incidence is higher in males(151).

Several studies demonstrated the presence of HCMV DNA, transcripts and/or proteins in GBM samples as already mentioned. In addition, it has also been shown that viral proteins can modulate the phenotype of GBM cells which could lead to cell proliferation(136), immortalization(61), migration(152,153), stem cell maintenance(111,154), angiogenesis(78,132,153,155) and immunomodulation(127). Although HCMV is not considered to be a classic oncogenic virus as it has not been proved to show acute transforming activity(156), it is possible that viral proteins may increase cellular malignancy and contribute to tumor progression, a phenotype called oncomodulation(157). Cinatl et al.(158) recommended the initial concept of oncomodulation as they suggested that HCMV could increase tumor malignancy by infecting tumor cells and affecting, directly or indirectly, various factors that contribute to tumorigenesis. In regards to GBM pathogenesis, it is considered that HCMV contributes to its pathogenesis through oncomodulation of host cellular pathways. That theory is based on evidence produced by studies carried out in mice models, and it was revealed that HCMV persistent infection of endothelial cells, which defined as the expression of viral genes without evidence of cytopathogenic effect on host cells, led to the production of inflammatory cytokines and renin, which could lead to hypertension development(159). Based on that suggestion, a hypothesis recommends that HCMV persistent infection could lead to production of inflammatory cytokines that may contribute to pathogenesis through disruption of the cell cycle(124). In a relevant HCM/ Gliomas Symposium it was
concluded that HCMV sequences and viral gene expression are present in many high-grade gliomas and that in vitro studies suggested that HCMV can modulate key signalling cellular pathways in GBMs(124).

HCMV encoded G protein-coupled receptor US28 expression which is a chemokine receptor, is implicated in GBM development as it has been suggested that modulates the HCMV-mediated oncogenesis. In cases of HCMV infection, US28 has constitutive activity which leads to G-protein dependent signalling (78). US28 chemokine is expressed in 60% of human GBM samples and can bind several chemokines such as CCL5,CCL2, and CX3CL1(78), whereas it regulates several cellular signaling pathways such as VEGF, STAT3, and endothelial nitric oxide synthase (e-NOS) which promotes GBM pathogenesis by regulating angiogenesis through PI3K/Akt pathway, invasion, and immune evasion(132,153). The activation of the PI3K/Akt signaling pathway was identified in cases of HCMV infection in human glioma tumor cells (152), its expression seems to increase glioma development(160), and it has also been found that US28-CCL5 paracrine signaling may contribute to glioma progression(153). US28 overexpression in glioma cells was associated with secretion of VEGF (78) due to activation of hypoxia inducible factor 1alpha/pyruvate kinase M2 (HIF-1alpha/PKM2) in GBM cells, lactate secretion(161), activation of STAT3, and an increased GBM cell invasiveness(162). HIF-1alpha/PKM2 inhibition could invert the increased proliferation of cells expressing US28(78).

Analysis of clinical GBM samples in situ showed colocalization of US28 with phosphorylated STAT3, COX-2, VEGF and e-NOS, whereas US28 could induce cellular migration in vitro, which suggests that US28 may contribute to tumour invasiveness and angiogenesis in vivo(60,69,132).

Cobbs et al. was found that HCMV IE1 protein promotes the tumor phenotype through inactivation of the tumor suppressor proteins p53 and Rb and through activation of the PI3-K/AKT signaling pathway(136). It has been investigated the effect of HCMV in tumor cells through the infection of cell lines. However, after transfection of glioma cell lines to overexpress the IE1 proteins, was found a decrease of tumor suppressor genes like p53 and downregulation of tumorigenic genes such as thrombospondin-1 (TSP-1)(163). Expression of HCMV IE1 genes in human GBM cell lines could increase entry into the cell cycle, synthesis of DNA, and cellular proliferation(136). On the contrary, Soroceanu et al. found that the attenuation of endogenous IE expression in glioma stem-like cells could inhibit development of the tumor cells. Moreover, cell lines without HCMV expression in case of their HCMV infection showed an increase in self-renewal and proliferation(162).

In another report was observed that long-term HCMV infected glioma cultures showed upregulation of signaling mediators such as SOX2, STAT3 which have oncogenic effects, and IL-6 which can affect all aspects of tumorigenesis process by regulating metabolism, survival, proliferation, angiogenesis, apoptosis, and metastasis(156).

HCMV pp71 protein induced stem cell factor (SCF) in GBM cells which is a proangiogenic factor, through nuclear factor NF-kB signaling pathway(155), whereas pp71 and NF-kB activation were found to be elevated in mesenchymal subtypes of human GBM samples. That protein is also implicated in reducing the accumulation of major histocompatibility complex (MHC) class I on the GBM cell surface(163). Moreover, overexpression of pp71 protein has been found to induce a pro-inflammatory response caused by the activation of NF-kB signaling pathway in adult neural precursor cells(155), and was preferably expressed in CD133+ glioma stem-like cells (155). HCMV shows a tropism for CD133 + glioma stem-like cells which produce HCMV IL-10. HCMV IL-10 induces the production of
immunosuppressive monocytes and expression of viral IE1. The immunosuppressive monocytes produce angiogenic factor VEGF, immunosuppressive TGFβ 1 and increased migration of glioma stem-like cells(127).

Fornara et al.(111) observed that infection of GBM cells with HCMV resulted in upregulation of stem cell markers like Sox 2, Notch 1, Oct4 and Nestin, and that HCMV infection induced neurosphere formation of GBM tumor cells.

HCMV glycoprotein B has been found to mediate viral cellular entry through the receptor tyrosine kinase PDGFR-α resulting in activation of the PI3-K/AKT signaling pathway(35). PDGFR-α phosphorylation consists a critical receptor for HCMV infection(164). Overexpression of HCMV glycoprotein B in glioma cells also induced entry into the cell cycle and increased invasiveness(35).

Costa et al. described an increase in arginase 2 (ARG2) in GBM cells infected with HCMV that expressed the IE proteins(165). ARG2 is implicated in nitric oxide metabolism. On the contrary, it has been found that HCMV down-regulates ARG2 in human GBM cells with a corresponding decrease in miRNA-613 expression, which was associated with GBM worse clinical course and prognosis (survival, tumor size, etc.)(166). Therefore, the implications of HCMV in glioma development may be partially immune mediated.

Another study by Yan Xing et al.(167) showed that HCMV infection led to glioma progression through an up-regulation of endocan, which also is known as endothelial cell-specific molecule-1, is a proteoglycan that is secreted by endothelial cells, is upregulated by proangiogenic factors, and the secretion of inflammatory cytokines, and has been shown to be involved in several cellular processes including angiogenesis.

HCMV infection induced phosphorylation of focal adhesion kinase (FAK) Tyr397, which is essential for integrin-mediated cell migration and invasion of glioma cells(152).

HCMV promotes tumor development and prevents apoptosis of GBM cells by overexpression of activating transcription factor 5 (ATF5). HCMV expression is associated with tumor proliferation, invasion, and increase of stem cell properties within glioma tumor cells(168).

In a study by Straat et al. the infection of malignant glioma cell lines with CMV resulted in activated telomerase in tumor cells(61), and suggested that this effect could be an association between viral infection and oncogenesis.

Factors associated with inflammatory macrophages have also been implicated in HCMV and GBM pathogenesis(127).

Pandey(169) investigated the mechanisms which are involved in the oncogenic potential of individual HCMV genes and could contribute to GBM pathogenesis, and suggested that the association remains controversial. In general, many previous studies showed that HCMV genes have the potential to be oncogenic; however the possible association between HCMV and GBM development remains unclear.

Several hypotheses have been carried out concerning that contingent association(36). At first HCMV was not considered to be a causal factor for GBM development. That concept has been proved only in a mouse model(170). The second hypothesis is that HCMV may be acts as an oncomodulatory virus, therefore
could be able to increase tumor progression by a specific path-way or a combination of pathways, which have already mentioned(35,60,111,124,132,136,139,155,156,171,172). Another hypothesis is that HCMV may be is present showing a little effect on tumor development, whereas its antigens are expressed due to the exceedingly immunosuppressive tumor microenvironment which exists in GBM, despite the fact that no direct evidence to date supports this hypothesis. The final hypothesis is that the mentioned observation consist an experimental artifact, as previous reports have described possible aspects in which HCMV detection could be attributed to cross-reactivity of antibodies(38), the concentration of antibodies(173), or presence of expression vector genetic material in sequencing datasets(90). Several studies suggest that extracellular factors released during HCMV infection can cause immunosuppression of the tumor microenvironment(174), but the virus may also be more directly involved in the malignant phenotype of tumor cells(175).

A possible role of HCMV would propose that the host immune system genes might mediate the neoplastic process in GBM and contribute to the supposed pathways for the development of GBM. However, the implication of those genes does not explain the highly significant differences in susceptibility to HCMV induced GBM, among individuals(176). The immunosuppression associated with GBM(177,178) would be the perfect environment for HCMV reactivation in tumor cells.

In conclusion, previous and recent researches for more than a decade have investigated the clinical relevance of HCMV infection in GBM pathogenesis. However, the association remains still unclear as there are discrepancies in the detection of HCMV and conflicting reports have been published regarding the ability to detect HCMV proteins and nucleic acids in GBM tissue. No current evidence exists for a potential role of HCMV in tumorigenesis, and especially in gliomagenesis, whereas it has been suggested that HCMV has possible oncomodulatory functions. Further multicenter and well-designed clinical studies, in which a standardized methodology must be used to determine whether this virus is actually present in glioma samples, and to clarify the potential role of HCMV direct, or indirect as an oncomodulator in GBM pathogenesis are required.

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