Оригінальна стаття

Ukr Neurosurg J. 2022;28(2):25-32
doi: 10.25305/unj.258544

Cytomegalovirus infection of brain tumors and CMV immunotherapy

Mykola I. Lisianyi, Antonina I. Klyuchnikova, Oleksandr M. Lisianyi, Liudmyla M. Belska, Larysa A. Kot, Diana M. Stanetska

Neuroimmunology Department, Romodanov Neurosurgery Institute, Kyiv, Ukraine

Received: 08 June 2022
Accepted: 12 August 2022

Address for correspondence*:*
Mykola I. Lisianyi, Neuroimmunology Department, Romodanov Neurosurgery Institute, 32 Platona Maiborody st., Kyiv, 04050, Ukraine, e-mail: nimun.neuro@gmail.com

Objective. The article presents the literature of the last ten years and the results of our own research on the importance of cytomegalovirus (CMV) in the development of brain tumors, especially glioblastoma and medulloblastoma. Two alternative views are discussed - the pros and cons of the role of the virus in the induction and stimulation of tumor growth.

Materials and methods. 256 samples of biotic material of tissues of various brain tumors were studied. Among them are histologically diagnosed: in 123 cases glial tumors of various grade of malignancy, in 51 cases meningiomas, in 25 cases medulloblastomas, in 16 cases oligodendroastrocytomas of the second grade of malignancy, in 14 cases metastatic tumors. Tumor fragments were obtained from biopsy material 1.5-2.0 hours after surgical removal. To detect the presence of CMV in the tumor tissue real-time polymerase chain reaction (PCR) using "DNA sorb A and B" kits was performed, the company "Amplisens" (Russia), according to the manufacturer's instructions and BioRal device (USA) with standard DNA detection kits of CMV "DNA Technology" (Russia). Cytological imprints on slides were also made from tumor tissue fragments, which were examined by indirect immunofluorescence method with monoclonal antibodies to CMV pP-65 protein using the "MonoScan CMV" kit.

Results. The frequency of detection of CMV antigen or its DNA in brain tissue depends on the research method - the immunofluorescence method detects pP-65 antigen by monoclonal antibodies 2-2.5 times more often than the PCR method of CMV in tumor tissue. In the tissue of different histogenesis of brain tumors both the pP-65 antigen and CMV DNA are detected with different frequencies. CMV was most often detected in tumors of glial origin and medulloblastomas. No CMV DNA was detected in the peripheral blood of patients with brain tumors at the time of admission for examination and surgical treatment, indicating an earlier contamination of the tumor focus with this virus. Data on the mechanisms of CMV induction and stimulation of tumor growth by activating cell proliferation, including nerve stem cells, are presented. Works using specific antiviral therapy and CMV specific cell immunotherapy in the treatment of gliomas have been analyzed in detail.

Conclusions. The paper concludes on the important clinical and prognostic value of determining CMV infection in brain tumors and indicates the need for CMV viral and cellular immunotherapy in the combined treatment of malignant brain tumors.

Key words: *cytomegalovirus; brain tumors; CMV immunotherapy*

Introduction

Among the methods of surgical and non-surgical combined treatment of human malignant tumors, in particular brain neoplasms, immunotherapy methods occupy an important place, which are considered to be the most promising and physiological method of fifghting malignant tumors. There are many immunotherapeutic methods of treatment, which are divided into specific and non-specific, active and passive, cellular and antigenic, infectious and molecular genetic, etc. Much attention is paid to the study of the possibility of creating highly active immunotherapeutic drugs, that would have high antigenicity due to adjuvants and viruses and the ability to cause a specific antitumor response in the body [1, 2]. Worthy of attention are researches on the study and creation of immunotherapeutic drugs from virus-infected tumors, which have both high antigenicity and a strong ability to stimulate cytotoxic activity of immune cells under conditions of tumor immunosuppression [1, 2].

Due to the use of molecular genetics and improvement of immunohistochemical research methods, a lot of data on the relationship between viruses and malignant human tumors (breast cancer, colon, prostate, salivary gland cancer, glioblastoma and medulloblastoma of the brain) have been obtained [2–6].

The assumption about the role of viruses, in particular cytomegalovirus (CMV), in the induction of human tumors has existed for a long time. Studies

conducted in the mid-1970s [7, 8] revealed the presence of CMV in tumors, but the role of this virus in oncogenesis has not been definitively elucidated. Accordingly, P.S. More and J. Chang in 2010 claimed that only 7 viruses are absolutely oncogenic and cause human tumors (10–15% of all neoplasms known in the world) [9]. According to these authors, oncology can be divided into infectious and non-infectious. Comparing viral infection and oncogenesis, many common characteristics can be found, such as inflammation, innate immune responses, immune suppression, etc. [9].

Despite the presence of viral antigens in the tumor, which are detected by immunohistological methods, the isolation and culture of CMV by classical virological methods *in vitro* is rarely possible, which forced supporters of the viral theory of oncogenesis to propose the "hit and run" hypothesis, which explained the frequent absence of the virus in tumor [10]. Later, the term "microinfection" of CMV in a tumor was proposed to explain the conflicting data on the role of the virus in the induction of tumors [11–13]. The reason for conflicting data is imperfect, outdated research methods. However, even the use of modern immunohistochemistry methods, genetic molecular methods such as polymerase chain reaction (PCR) not always allowed to detect CMV infection in tumors [14]. The work of German scientists who used modern methods to investigate 22 glial tumors, 6 breast and colon adenocarcinomas, 6 lung tumors, and 4 normal brain tissue samples did not detect CMV in the tumor tissue is of interest [15]. The theory of the etiological role of CMV in the oncogenesis of malignant brain tumors is not recognized by all researchers and requires further study.

Proponents of the CMV-theory of neuro-oncogenesis provide a lot of evidence in its favor. Thus, it has been shown that US-28, a CMV chemokine receptor, can bind chemokine receptors of cells, which leads to their activation and stimulation of proliferation and synthesis of proangiogenic factors, in particular, vascular endothelial growth factor. Injection of glial cells expressing US-28 protein to mice led to the development of tumors in them [16] and activated the transcription factor of cell transduction and activation – STAT-3 [17]. It has also been shown that immediate-early proteins of CMV encoded by 23/122 genes responsible for viral replication, can induce glioblastoma growth [18]. On glioblastoma cell lines, ambiguous results of detection of these genes have been obtained. Thus, the expression of these genes in some cell lines caused increased cell proliferation, while in others it inhibited their proliferation, blocking cell division [19]. CMV infection has been shown to induce phosphorylation of intracellular kinases, and CMV IE-2 protein combines with histone deacetylase-2, which enhances the transcriptional activity of cells [20]. In the cell, CMV infection can bind to micro-RNAs that play an important role in tumor induction [21, 22].

From the following data, it can be concluded that the presence of CMV in gliomas is not a random laboratory phenomenon caused by contamination with tumor viral antigens, CMV plays an important role, if not in tumor induction, then at least in oncomodulatory action. The influence of CMV on other intratumoral processes, in particular, its role in the development of intratumoral immunosuppression is not clear. It is also not known whether the suppression creates conditions for the development and preservation of CMV in the tumor or, on the contrary, CMV infection induces immunosuppression in the tumor and thus leads to tumor growth and inhibition of antitumor immunity. Thus, CMV pp-65 protein, which quantitatively prevails among the virus proteins, is able to suppress the activity of natural killer (NK cells) and γ-interferon synthesis, destroying HLA-DR molecules on lymphocytes membrane [23]. CMV-infected blood monocytes begin to synthesize the so-called CMV-dependent interleukin (IL)-10, which binds to the corresponding receptor on the cell and activates the transcription factor STAT-3 [24,25], which is a key molecule in carcinogenesis and immune suppression in tumor [26].

STAT-3 factor is known to be important for the activation and migration of neural stem cells (NSCs) [28, 29]. Moreover, CMV-induced IL-10 suppresses the synthesis of pro-inflammatory cytokines [30] and inhibits the proliferation of monocyte progenitors [31]. This factor leads to the transformation of pro-inflammatory M1 monocytes into immunosuppressive M2 monocytes, which suppress immune cells functions [32,33]. The presence of M2 monocytes in the tumor is considered to be an unfavorable prognostic sign [33]. These data indicate that CMV infection in the tumor focus can be not only a direct etiological cause of tumor development, but also indirectly affect the growth of glioma through immune mechanisms. CMV binds to NSCs and monocytes, which synthesize IL-10 and other factors that cause immunosuppression and the formation of M2 monocytes, as well as affect angiogenesis in the tumor, activate STAT-3 and stimulate the proliferation of tumor cells [1, 2].

There are two stages of interaction between CMV and the tumor. At the first stage, CMV binds to NSCs through the platelet-derived growth factor receptor and activates the STAT-3 factor in them, which ensures the migration of these cells and the synthesis of IL-10. The latter affects monocytes, turning them into M2 cells. The second stage is associated with M2 cells that accumulate in the tumor focus and indirectly stimulate tumor growth by affecting angiogenesis, immune response, migration and invasion of tumor cells [1, 28, 34].

It can be argued that the interaction of CMV and body cells in which this virus persists can lead to tumor induction or its oncomodulation implemented by various intracellular mechanisms that cause the blockade and inhibition of some processes and the stimulation of others ***(Fig. 1).***

According to modern theories of oncogenesis, glioblastomas and medulloblastomas develop from stem tumor cells [34,35], which may arise as a result of impaired normal differentiation of NSCs into astrocytes under the influence of mutagenic factors and viruses [36]. Thus, brain NSCs are known to express the platelet-derived growth factor receptor, which binds to one of the CMV gB proteins, which leads to virus penetration into stem cells and its reproduction in the cell followed by activation of the phosphoinositidine-3-kinase (PI3K) signaling pathway, which is responsible for proliferation processes [37]. It has also been shown that CMV can infect NSCs of healthy individuals. Given

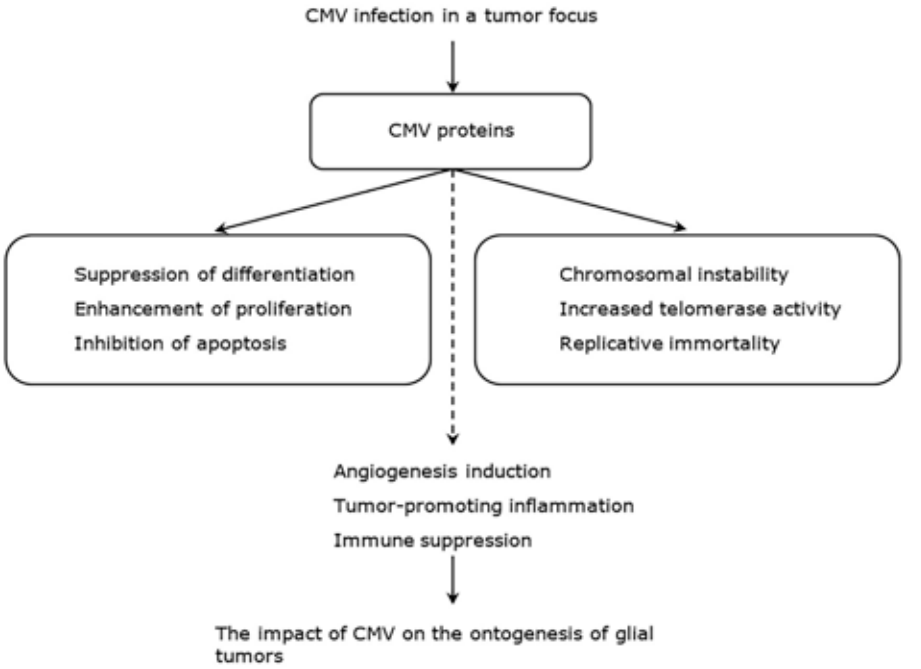

Fig. 1. The effect of cytomegalovirus proteins on cellular processes that can cause tumor induction or stimulation [3]

their pluripotent properties, the possibility of their transformation into tumor stem cells containing CMV cannot excluded [38]. The hypothesis about the role of CMV in NSCs infection in glioblastoma oncogenesis is confirmed by the fact that binding cultures of glioblastoma cells, in which no tumor stem cells are detected, do not contain CMV as well, and, on the contrary, glioblastoma cell cultures, in which NSCs are present, contain CMV proteins. Additional indirect evidence of association between CMV and NSCs is that these cells are located in the subventricular zone of the brain, where CMV can persist for a long time, as was found in mice infected with this virus [39].

It is possible that the microenvironment in the tumor focus (macrophage-monocytic cells and NSCs) can be a reservoir of the virus in the tumor [40].

Despite clinical and theoretical studies of the relationship between CMV and neuro-oncogenesis, there is still skepticism about the presence and role of CMV in the induction of malignant brain tumors [14].

Purpose: to investigate the role of cytomegalovirus (CMV) in the induction and stimulation of brain tumor growth.

Materials and methods

Study participants

256 samples of biotic material from various brain tumors of patients operated on at the Institute of Neurosurgery named after Acad. A.P. Romodanov, Ukraine in 2014–2017 were studied.

Informed and voluntary written consent to participate in the study was obtained from all patients.

The study was approved by the Committee on Ethics and Bioethics of the Institute of Neurosurgery named after Acad. A. P. Romodanov, Ukraine (Minutes № 1 dated April 14, 2013).

Inclusion criteria

Age of patients is from 16 to 55 years, absence of chronic diseases (tuberculosis, diabetes), immunodeficiency and allergic diseases, viral hepatitis and other infectious diseases.

Characteristics of groups

Among 256 tumors, histologically according to the WHO international classification of tumors of the central nervous system (2007), 123 glial tumors of various grade of anaplasia, 51 meningiomas, 25 medulloblastomas, 16 oligodendroastrocytomas of grade II of anaplasia, 14 metastatic tumors were diagnosed.

Study design

The tumor fragments obtained from biopsy material 1.5–2.0 h after surgical removal were used. To detect the presence of DNA virus in the tumor tissue, a real-time molecular genetic PCR method was used utilizing DNA-sorb A and B kits (Amplisens, RF) according to the manufacturer's instructions and the BioRal device (USA) with standard kits for determining CMV DNA ("DNA-technology", RF). Samples in which the PCR reaction occurred before the 36th cycle, as specified in the manufacturer's instructions, were considered positive ones. Cytological imprints were also obtained from tumor tissue fragments on glass slides, which, after drying and fixing with 96% ethanol, were examined by the indirect immunofluorescence method with monoclonal antibodies to pP-65 of the CMV protein using the "MonoScan CMV" kit (RF).

In the blood of 60 neuro-oncology patients hospitalized for surgical treatment, in the preoperative period, the presence of CMV DNA was determined by real-time PCR.

Statistical analysis

Statistical data processing was carried out using the Microsoft Excel software package with the determination of Student's t-test.

Results and discussion

The immunofluorescent study of CMV pP-65 protein content in brain tumor cells of different histostructures, revealed that pP-65 protein expression in tumor cells varies in a wide range: 80.0% (20 out of 25) – in medulloblastomas, 68.7% (114 out of 166) - in gliomas, 65.0% (13 out of 20) - in meningiomas, 35.7% 5 out of 14, $p<0.05$ compared to other groups of tumors) - in cancer metastases, 60.9% 14 out of 23) – in other tumors.

Among glial tumors, pP-65 protein was detected in 68.7% of the studied samples, in glioblastomas and anaplastic astrocytomas - 1.5–2.0 times more often than in differentiated benign astrocytomas (in 76.2% (48 out of 63) glioblastomas grade IV of anaplasia, 66.3% (57 out of 86) anaplastic astrocytoma grade III of anaplasia, 52.0% (9 out of 17) fibrillary protoplasmic astrocytoma grade II of anaplasia). The difference between the glioblastoma group and the fibrillary protoplasmic astrocytic group was statistically significant ($p<0.05$). In no case was the presence of pP-65 protein detected in normal brain matter.

Therefore, it can be concluded that there is a significant persistence of CMV pP-65 protein in brain gliomas of different histostructure and grade of anaplasia.

PCR study of the presence of viral DNA in tumor tissue yielded somewhat different results. CMV DNA was detected in only 23.8% of the samples, which is three times less compared to the determination of CMV pP-65 protein by the immunofluorescence method. In brain tumors of different histogenesis, CMV DNA was detected with different frequencies. Most often, DNA was determined in tumors of glial origin (33.3% (41 out of 123)), slightly less in medulloblastomas and other tumors (20.0% (5 out of 225) and 18.75% (6 out of 32), respectively). In meningiomas arising from the meninges, CMV was detected in only 5.88% (3 out of 51) of observations ($p<0.05$ compared to other tumor groups). CMV DNA was not detected in any sample of normal brain matter. Our data on the frequency of CMV persistence in brain tumors are consistent with literature data on the predominant CMV contamination of glial tumors [5, 6].

Thus, CMV can be determined by the presence of both antigens and viral DNA in tumor cells. In other primary and metastatic brain tumors, viral proteins, in particular pP-65, are detected more frequently, and viral DNA is detected less often, which probably indicates the short-term persistence of the virus in these tumors. Systemic activation of chronic CMV infection in patients with brain tumors and mechanical transient transmission of the virus from the blood to tumor vessels and the tumor focus are also not excluded.

To diagnose the activation of chronic CMV infection, 60 peripheral blood samples of patients with brain tumors at the time of hospitalization for examination and surgical treatment were studied. The frequency of viral DNA detection in peripheral blood was 6% (one observation each in glioblastoma, medulloblastoma, pituitary adenoma, and meningioma) [41].

The findings reveal the absence of activation of latent CMV infection in patients with brain tumors at the time of hospitalization, as well as the fact that the contamination of CMV tumors occurred much earlier, and the detection of viral proteins and DNA in tumor tissue cells is not a consequence of transient or mechanical transmission of the virus from the blood to the tumor focus.

The mechanism of virus activation and transmission from the blood to the tumor focus and their long-term or short-term stay in tumor tissue has been little studied, although many authors indicate the important role in these processes of specific antiviral immunity, the suppression of which can lead to the activation of chronic infections, in particular CMV.

The clinical significance of the phenomenon of CMV protein expression in brain tumors has not been sufficiently investigated. There are only single studies that analyze the life expectancy of patients and the presence of CMV infection. The most complete analysis of survival of 80 patients with glioblastomas is given in the work of H. Rahbar *et al.*, (2012) [42]. The patients were divided into two groups: with life expectancy of up to 18 months (40 people) and over 18 months (40 people). The following pattern has been established: the lower CMV expression in the tumor, the longer the patient's life expectancy. When analyzing the data, a number of factors (chemotherapy, radiosurgical treatment, tumor localization and the volume of surgical intervention) as well as other reasons that can significantly affect the life expectancy of patients should be taken into account. The authors, performing a complex statistical analysis, concluded that a low content of CMV-infected tumor cells is a favorable prognostic indicator determining the life expectancy of patients, and vice versa, a high level of CMV in tumor tissue is an unfavorable prognostic sign. For a final conclusion, additional studies on the possibility of predicting life expectancy based on infection of CMV tumors should be to carried out [42].

CMV has previously been shown to induce the synthesis of tumor cyclooxygenase-2 (COX-2) and prostaglandin E-2 (PGE-2) [39]. A high level of COX-2 was found in some samples of medulloblastomas and glioblastomas, which is associated with a poor clinical prognosis [39, 43, 44]. The use of the COX-2 inhibitor and cellotoxit in combination with velorin (Cymevene) contributed to the inhibition of medulloblastoma growth by 40–50% in an experiment on mice, and the content of CMV proteins in this case decreased by 80% in the tissue of xenografts of these tumors [43, 45]. It is possible that the combined use of antiviral therapy and COX-2 inhibitors in the clinic will provide a positive result in the treatment of CMV tumors. The first attempts to treat patients with gliomas with such therapy were made. More than 40 patients with gliobastomas receive antiviral and antiCOX-2 therapy in combination with radiological therapy and chemotherapy in addition to traditional treatment [46, 47]. However, the issue of specific antiviral treatment (cymevene, ganciclovir, etc.) of patients with glioblastomas remains controversial, despite a significant number of publications [46–48]. Thus, a retrospective survival analysis of 50 patients with glioblastomas who received ganciclovir in addition to standard treatment showed that their overall survival was 25.0 months compared to 13.5 months in the control

group (p<0.001) [48]. A small study comparing treatment with a combination of ganciclovir and bevacizumab in relapsed glioblastoma with bevacizumab alone showed only a trend toward improved overall survival (13.1 and 8.7 months, respectively) [49].

Thus, the benefit of CMV-specific antiviral treatment for glioblastomas is questionable, so the use of antiviral treatment in patients with glioblastomas is not a routine therapy and requires both experimental and clinical studies.

Another methodological approach, namely the use of CMV as a useful target in the immunotherapeutic treatment of malignant tumors, continues to be investigated. K. G. Lucas *et al*., were the first to discover that CMV-specific T cells are able to recognize and lyse CMV-infected glioblastoma cells *in vitro* [49]. Immunotherapeutic strategies for targeting immune cells to CMV include techniques such as adoptive T-cells transfer and CMV-based vaccines. Adoptive T-cell therapy has been primarily studied using CMV-specific T-cells from patients with gliomas that have been activated *ex vivo*. T-cells were found to function poorly in *ex vivo* assays. However, their functionality was restored *in vitro* with the help of CMV peptide antigens and exposure to IL-2 [50]. T-cells specific to the CMV antigen can be isolated from the blood of patients with glioblastomas [51]. They can proliferate and become activated by CMV antigens and dendritic cells. T-cells induced a potent antitumor antigen-specific response only *in vitro* when co-cultured with autologous glioma tumor cells. These results demonstrated that the endogenous level of CMV antigens in glioma cells shold be high enough to be a target for CMV-specific T-cells induced *in vitro*. In a study with recurrent glioblastoma, such CMV-specific T-cells were injected to 11 patients as intravenous infusions in combination with chemotherapy. Overall survival was short - only 13.4 months [50]. For the first time in 2014, A. Schuessler *et al*., completed phase I clinical trial of CMV immunotherapy. They gave 13 patients with recurrent glioblastoma 3-4 intravenous infusions of autologous CMV-specific T-cells in doses from 25 to 40 · 10^6. CMV-specific therapy was found to increase median overall survival (>57 weeks), median progression-free survival (>35 weeks) and progression-free rate of 40% after the first relapse with mild to moderate complications (headache or fatigue), none of which were serious [52]. The stage of the disease affects the effectiveness of CMV immunotherapy. Thus, the administration of CMV-specific T cells to patients before tumor recurrence, and not after it, resulted in a 2.5-fold life prolongation (10 and 4 months, respectively) and median overall survival of 23 and 14 months [53]. Moreover, SK Nair *et al*., in a preclinical study suggested that a patient's autologous T-cells stimulated by autologous dendritic cells (DCs) can stimulate the proliferation and CMV pP65-specific T cells 10-20-times with a significant increase in the level of cytotoxic interferon-γ + /CD + /CD8 + cells [54]. It was found that a single intravenous injection of 3 · 10^7 CMV pP65-specific T-cells/kg in combination with three intradermal infusions of 2 · 10^7 DC compared to the control group was found to have a significant effect on the cytotoxic activity of CMV-specific CD-8 lymphocytes and cytokine synthesis [55].

The effectiveness of immunotherapy against CMV may be due to several mechanisms [1, 2, 48]: directing the immune response to glioma cells expressing CMV (direct antigen-specific hypothesis) and activation of other immune cells leading to additional destruction of CMV-negative cells (hypothesis of indirect cytotoxicity) with cross-priming of immune cells after destruction of CMV-positive tumor cells (cross-priming).

The essence of the antigen-specific hypothesis regarding the role of CMV immunotherapy in the induction of a cytotoxic immune response against CMV, leading to the specific cytotoxic death of CMV-positive tumor cells, especially CMV-positive tumor stem cells, and inhibition of tumor progression [35, 36, 38, 39].

Indirect cytotoxicity hypothesis, based on strong immune activation in the tumor microenvironment, when natural killers, macrophages and memory T cells join the antigen-specific immune response, and an increase in the synthesis of pro-inflammatory cytokines is also observed [56, 57]. These recruited immune effector cells can cause tumor cell destruction independent of the presence of CMV in the tumor, which leads to the development of a specific antitumor T-cell response that is able to control tumorigenesis and tumor growth [2, 56].

When CMV-specific immune cytotoxic destruction of tumor cells leads to the release of cellular and viral antigens from apoptotic and necrotic cells and cross-priming and DC activation of the immune system by these antigens. These cells induce an immune response and thus enhance the antitumor immune response and lead to the destruction of both CMV-positive and CMV-negative tumor cells.

Experimental and clinical results of studies using modern CMV immunotherapy strategies are probably associated with the simultaneous action of these mechanisms, but many issues remain unexplored, such as the role and state of the blood-brain barrier during immunotherapy, heterogeneity and low level of protein expression CMV in tumor cells, combination of CMV immunotherapy with chemotherapy or radiation therapy, etc.

Studies of CMV immunotherapy in glioma have shown that CMV viral material can be detected in tumor cells and viral proteins can be potent targets for immunotherapy due to their foreign antigen nature. A comprehensive study of CMV infection in malignant brain tumors and improvement of CMV immunotherapy drugs will allow to use this method more widely in the combined treatment of tumors.

It is known that CMV can persist in human non-glial malignant tumors as well [3–7]. Probably, this is a universal property of this virus to accompany any malignant processes in the body. In other words, it is only about the association of CMV with a malignant tumor process in the body. Tumors containing CMV are recommended to be called "CMV-associated glioblastomas or medulloblastomas" [40]. The possibility of using this association for prognosis or treatment of malignant brain tumors has not been adequately studied. It is believed that CMV immunotherapy is a promising direction in the development of modern methods of treating brain tumors.

Conclusions

1. The frequency of detection of CMV antigen or its DNA in brain tumor tissue depends on the research method: the immunofluorescence method detects pP-65 protein using monoclonal antibodies 2.0–2.5 times more often than the CMV PCR method in tumor tissue, which gives the grounds to recommend these methods for diagnosis in clinical practice.

2. Both CMV pP-65 protein and CMV DNA are determined with varying frequency in the tissue of brain tumors of different histogenesis. Most often, CMV DNA was detected in tumors of glial origin and medulloblastomas, which indirectly indicates the possible role of the virus in the development of malignant tumors.

3. CVM DNA is not detected in the peripheral blood of patients with brain tumors at the time of hospitalization for examination and surgical treatment, which indicates a long-term persistence of the virus in the tumor focus and earlier contamination of tumors with this virus.

4. The obtained data indicate both the possible etiological or tumor-stimulating role of CMV in the development of brain tumors, especially malignant gliomas, and the prognostic value of determining the degree of CMV infection of tumor cells.

5. There are different methods of CMV immunotherapy of brain tumors. It is necessary to use the methods of antiviral CMV therapy more widely in the combined treatment of patients with malignant brain tumors, especially if CMV DNA and proteins are detected in the tumor.

Disclosure

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed and voluntary written consent to participate in the study was obtained from all patients.

The study was approved by the Committee on Ethics and Bioethics of the Institute of Neurosurgery named after Acad. A. P. Romodanov, Ukraine (Minutes № 1 dated April 14, 2013).

Funding

The study was not sponsored.

References

1. Rahman M, Dastmalchi F, Karachi A, Mitchell D. The role of CMV in glioblastoma and implications for immunotherapeutic strategies. Oncoimmunology. 2018 Oct 16;8(1):e1514921. doi: 10.1080/2162402X.2018.1514921
2. Daei Sorkhabi A, Sarkesh A, Saeedi H, Marofi F, Ghaebi M, Silvestris N, Baradaran B, Brunetti O. The Basis and Advances in Clinical Application of Cytomegalovirus-Specific Cytotoxic T Cell Immunotherapy for Glioblastoma Multiforme. Front Oncol. 2022 Apr 19;12:818447. doi: 10.3389/fonc.2022.818447
3. Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? Virus Res. 2011 May;157(2):193-203. doi: 10.1016/j.virusres.2010.10.026
4. Melnick M, Sedghizadeh PP, Allen CM, Jaskoll T. Human cytomegalovirus and mucoepidermoid carcinoma of salivary glands: cell-specific localization of active viral and oncogenic signaling proteins is confirmatory of a causal relationship. Exp Mol Pathol. 2012 Feb;92(1):118-25. doi: 10.1016/j.yexmp.2011.10.011
5. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG, Britt WJ. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res. 2002 Jun 15;62(12):3347-50.
6. Scheurer ME, Bondy ML, Aldape KD, Albrecht T, El-Zein R. Detection of human cytomegalovirus in different histological types of gliomas. Acta Neuropathol. 2008 Jul;116(1):79-86. doi: 10.1007/s00401-008-0359-1
7. Geder L, Sanford EJ, Rohner TJ, Rapp F. Cytomegalovirus and cancer of the prostate: in vitro transformation of human cells. Cancer Treat Rep. 1977 Mar-Apr;61(2):139-46.
8. Sanford EJ, Geder L, Laychock A, Rohner TJ Jr, Rapp F. Evidence for the association of cytomegalovirus with carcinoma of the prostate. J Urol. 1977 Nov;118(5):789-92. doi: 10.1016/s0022-5347(17)58194-x
9. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2010 Dec;10(12):878-89. doi: 10.1038/nrc2961
10. Shen Y, Zhu H, Shenk T. Human cytomagalovirus IE1 and IE2 proteins are mutagenic and mediate "hit-and-run" oncogenic transformation in cooperation with the adenovirus E1A proteins. Proc Natl Acad Sci USA. 1997 Apr 1;94(7):3341-5. doi: 10.1073/pnas.94.7.3341
11. Cinatl J Jr, Vogel JU, Kotchetkov R, Wilhelm Doerr H. Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. FEMS Microbiol Rev. 2004 Feb;28(1):59-77. doi: 10.1016/j.femsre.2003.07.005
12. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol. 2003 Sep;170(3):998-1002. doi: 10.1097/01.ju.0000080263.46164.97
13. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, Cobbs CS. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002 Nov 16;360(9345):1557-63. doi: 10.1016/S0140-6736(02)11524-8
14. Poltermann S, Schlehofer B, Steindorf K, Schnitzler P, Geletneky K, Schlehofer JR. Lack of association of herpesviruses with brain tumors. J Neurovirol. 2006 Apr;12(2):90-9. doi: 10.1080/13550280600654573
15. Lau SK, Chen YY, Chen WG, Diamond DJ, Mamelak AN, Zaia JA, Weiss LM. Lack of association of cytomegalovirus with human brain tumors. Mod Pathol. 2005 Jun;18(6):838-43. doi: 10.1038/modpathol.3800352
16. Maussang D, Verzijl D, van Walsum M, Leurs R, Holl J, Pleskoff O, Michel D, van Dongen GA, Smit MJ. Human cytomegalovirus-encoded chemokine receptor US28 promotes tumorigenesis. Proc Natl Acad Sci USA. 2006 Aug 29;103(35):13068-73. doi: 10.1073/pnas.0604433103
17. Slinger E, Maussang D, Schreiber A, Siderius M, Rahbar A, Fraile-Ramos A, Lira SA, Söderberg-Nauclér C, Smit MJ. HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. Sci Signal. 2010 Aug;3(133):ra58. doi: 10.1126/scisignal.2001180
18. Sorg G, Stamminger T. Strong conservation of the constitutive activity of the IE1/2 transcriptional control region in wild-type strains of human cytomegalovirus. J Gen Virol. 1998 Dec;79 (Pt 12):3039-47. doi: 10.1099/0022-1317-79-12-3039
19. Cobbs CS, Soroceanu L, Denham S, Zhang W, Kraus MH. Modulation of oncogenic phenotype in human glioma cells by cytomegalovirus IE1-mediated mitogenicity. Cancer Res. 2008 Feb 1;68(3):724-30. doi: 10.1158/0008-5472.CAN-07-2291
20. Park JJ, Kim YE, Pham HT, Kim ET, Chung YH, Ahn JH. Functional interaction of the human cytomegalovirus IE2 protein with histone deacetylase 2 in infected human fibroblasts. J Gen Virol. 2007 Dec;88(Pt 12):3214-3223. doi: 10.1099/vir.0.83171-0
21. Kluiver J, Kroesen B, Poppema S, van den Berg A. The role

of microRNAs in normal hematopoiesis and hematopoietic malignancies. Leukemia. 2006 Nov;20(11):1931-6. doi: 10.1038/sj.leu.2404387
22. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T, Holland EC. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev. 2009 Jun;23(11):1327-37. doi: 10.1101/gad.1777409
23. Popkin DL, Watson MA, Karaskov E, Dunn GP, Bremner R, Virgin HW 4th. Murine cytomegalovirus paralyzes macrophages by blocking IFN gamma-induced promoter assembly. Proc Natl Acad Sci USA. 2003 Nov;100(24):14309-14. doi: 10.1073/pnas.1835673100
24. Wehinger J, Gouilleux F, Groner B, Finke J, Mertelsmann R, Weber-Nordt RM. IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. FEBS Lett. 1996 Oct 7;394(3):365-70. doi: 10.1016/0014-5793(96)00990-8
25. Spencer JV, Cadaoas J, Castillo PR, Saini V, Slobedman B. Stimulation of B lymphocytes by cmvIL-10 but not LAcmvIL-10. Virology. 2008 Apr 25;374(1):164-169. doi: 10.1016/j.virol.2007.11.031
26. Abou-Ghazal M, Yang DS, Qiao W, Reina-Ortiz C, Wei J, Kong LY, Fuller GN, Hiraoka N, Priebe W, Sawaya R, Heimberger AB. The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. Clin Cancer Res. 2008 Dec 15;14(24):8228-35. doi: 10.1158/1078-0432.CCR-08-1329
27. Brantley EC, Benveniste EN. Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas. Mol Cancer Res. 2008 May;6(5):675-84. doi: 10.1158/1541-7786.MCR-07-2180
28. Sherry MM, Reeves A, Wu JK, Cochran BH. STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. Stem Cells. 2009 Oct;27(10):2383-92. doi: 10.1002/stem.185
29. Wei J, Barr J, Kong LY, Wang Y, Wu A, Sharma AK, Gumin J, Henry V, Colman H, Priebe W, Sawaya R, Lang FF, Heimberger AB. Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. Mol Cancer Ther. 2010 Jan;9(1):67-78. doi: 10.1158/1535-7163.MCT-09-0734
30. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol. 2002 Feb;76(3):1285-92. doi: 10.1128/jvi.76.3.1285-1292.2002
31. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. Nat Neurosci. 2007 Dec;10(12):1544-53. doi: 10.1038/nn2015
32. O'Farrell AM, Liu Y, Moore KW, Mui AL. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. EMBO J. 1998 Feb 16;17(4):1006-18. doi: 10.1093/emboj/17.4.1006
33. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res. 2006 Dec 1;66(23):11238-46. doi: 10.1158/0008-5472.CAN-06-1278
34. Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, Alvarez-Buylla A, Parada LF. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell. 2009 Jan 6;15(1):45-56. doi: 10.1016/j.ccr.2008.12.006
35. Luo MH, Schwartz PH, Fortunato EA. Neonatal neural progenitor cells and their neuronal and glial cell derivatives are fully permissive for human cytomegalovirus infection. J Virol. 2008 Oct;82(20):9994-10007. doi: 10.1128/JVI.00943-08
36. Odeberg J, Wolmer N, Falci S, Westgren M, Sundtröm E, Seiger A, Söderberg-Nauclér C. Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes. J Neurosci Res. 2007 Feb 15;85(3):583-93. doi: 10.1002/jnr.21144
37. Soroceanu L, Akhavan A, Cobbs CS. Platelet-derived growth factor-alpha receptor activation is required for human cytomegalovirus infection. Nature. 2008 Sep 18;455(7211):391-5. doi: 10.1038/nature07209
38. Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C. The detection of CMV pp65 and IE1 in glioblastoma multiforme. J Neurooncol. 2011 Jun;103(2):231-8. doi: 10.1007/s11060-010-0383-6
39. Söderberg-Nauclér C. HCMV microinfections in inflammatory diseases and cancer. J Clin Virol. 2008 Mar;41(3):218-23. doi: 10.1016/j.jcv.2007.11.009
40. Ranganathan P, Clark PA, Kuo JS, Salamat MS, Kalejta RF. Significant association of multiple human cytomegalovirus genomic Loci with glioblastoma multiforme samples. J Virol. 2012 Jan;86(2):854-64. doi: 10.1128/JVI.06097-11
41. Lisyaniy AN. [Antibodies to cytomegalovirus in blood serum and virus pp65 in neoplastic cells of patients with brain tumors]. Onkologiya. 2013;15(2):108-112. Russian.
42. Rahbar A, Stragliotto G, Orrego A, Peredo I, Taher C, Willems J, Söderberg-Naucler C. Low levels of Human Cytomegalovirus Infection in Glioblastoma multiforme associates with patient survival; -a case-control study. Herpesviridae. 2012 Mar 16;3:3. doi: 10.1186/2042-4280-3-3
43. Baryawno N, Rahbar A, Wolmer-Solberg N, Taher C, Odeberg J, Darabi A, Khan Z, Sveinbjörnsson B, FuskevÅg OM, Segerström L, Nordenskjöld M, Siesjö P, Kogner P, Johnsen JI, Söderberg-Nauclér C. Detection of human cytomegalovirus in medulloblastomas reveals a potential therapeutic target. J Clin Invest. 2011 Oct;121(10):4043-55. doi: 10.1172/JCI57147
44. Johnsen JI, Baryawno N, Söderberg-Nauclér C. Is human cytomegalovirus a target in cancer therapy? Oncotarget. 2011 Dec;2(12):1329-38. doi: 10.18632/oncotarget.383
45. Söderberg-Nauclér C, Johnsen JI. Cytomegalovirus infection in brain tumors: A potential new target for therapy? Oncoimmunology. 2012 Aug 1;1(5):739-740. doi: 10.4161/onci.19441
46. Hadaczek P, Ozawa T, Soroceanu L, Yoshida Y, Matlaf L, Singer E, Fiallos E, James CD, Cobbs CS. Cidofovir: a novel antitumor agent for glioblastoma. Clin Cancer Res. 2013 Dec 1;19(23):6473-83. doi: 10.1158/1078-0432.CCR-13-1121
47. Stragliotto G, Rahbar A, Solberg NW, Lilja A, Taher C, Orrego A, Bjurman B, Tammik C, Skarman P, Peredo I, Söderberg-Nauclér C. Effects of valganciclovir as an add-on therapy in patients with cytomegalovirus-positive glioblastoma: a randomized, double-blind, hypothesis-generating study. Int J Cancer. 2013 Sep 1;133(5):1204-13. doi: 10.1002/ijc.28111
48. Söderberg-Naucler C, Peredo I, Rahbar A, Hansson F, Nordlund A, Stragliotto G. Use of Cox regression with treatment status as a time-dependent covariate to re-analyze survival benefit excludes immortal time bias effect in patients with glioblastoma who received prolonged adjuvant treatment with valganciclovir. Int J Cancer. 2014 Jul 1;135(1):248-9. doi: 10.1002/ijc.28663
49. Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C. The detection of CMV pp65 and IE1 in glioblastoma multiforme. J Neurooncol. 2011 Jun;103(2):231-8. doi: 10.1007/s11060-010-0383-6
50. Crough T, Beagley L, Smith C, Jones L, Walker DG, Khanna R. Ex vivo functional analysis, expansion and adoptive transfer of cytomegalovirus-specific T-cells in patients with glioblastoma multiforme. Immunol Cell Biol. 2012 Oct;90(9):872-80. doi: 10.1038/icb.2012.19
51. Nair SK, De Leon G, Boczkowski D, Schmittling R, Xie W, Staats J, Liu R, Johnson LA, Weinhold K, Archer GE, Sampson JH, Mitchell DA. Recognition and killing of autologous, primary glioblastoma tumor cells by human cytomegalovirus pp65-specific cytotoxic T cells. Clin Cancer Res. 2014 May 15;20(10):2684-94. doi: 10.1158/1078-0432.CCR-13-3268
52. Schuessler A, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, Jones L, Crough T, Dasari V, Klein K, Smalley A, Alexander H, Walker DG, Khanna R. Autologous T-cell therapy for cytomegalovirus as a consolidative

treatment for recurrent glioblastoma. Cancer Res. 2014 Jul 1;74(13):3466-76. doi: 10.1158/0008-5472.CAN-14-0296
53. Smith C, Lineburg KE, Martins JP, Ambalathingal GR, Neller MA, Morrison B, Matthews KK, Rehan S, Crooks P, Panikkar A, Beagley L, Le Texier L, Srihari S, Walker D, Khanna R. Autologous CMV-specific T cells are a safe adjuvant immunotherapy for primary glioblastoma multiforme. J Clin Invest. 2020 Nov 2;130(11):6041-6053. doi: 10.1172/JCI138649
54. Nair SK, De Leon G, Boczkowski D, Schmittling R, Xie W, Staats J, Liu R, Johnson LA, Weinhold K, Archer GE, Sampson JH, Mitchell DA. Recognition and killing of autologous, primary glioblastoma tumor cells by human cytomegalovirus pp65-specific cytotoxic T cells. Clin Cancer Res. 2014 May 15;20(10):2684-94. doi: 10.1158/1078-0432.CCR-13-3268
55. Reap EA, Suryadevara CM, Batich KA, Sanchez-Perez L, Archer GE, Schmittling RJ, Norberg PK, Herndon JE 2nd, Healy P, Congdon KL, Gedeon PC, Campbell OC, Swartz AM, Riccione KA, Yi JS, Hossain-Ibrahim MK, Saraswathula A, Nair SK, Dunn-Pirio AM, Broome TM, Weinhold KJ, Desjardins A, Vlahovic G, McLendon RE, Friedman AH, Friedman HS, Bigner DD, Fecci PE, Mitchell DA, Sampson JH. Dendritic Cells Enhance Polyfunctionality of Adoptively Transferred T Cells That Target Cytomegalovirus in Glioblastoma. Cancer Res. 2018 Jan 1;78(1):256-264. doi: 10.1158/0008-5472.CAN-17-0469
56. Ma HL, Whitters MJ, Konz RF, Senices M, Young DA, Grusby MJ, Collins M, Dunussi-Joannopoulos K. IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-gamma. J Immunol. 2003 Jul 15;171(2):608-15. doi: 10.4049/jimmunol.171.2.608
57. Dhanji S, Teh HS. IL-2-activated CD8+CD44high cells express both adaptive and innate immune system receptors and demonstrate specificity for syngeneic tumor cells. J Immunol. 2003 Oct 1;171(7):3442-50. doi: 10.4049/jimmunol.171.7.3442
58. Tietze JK, Wilkins DE, Sckisel GD, Bouchlaka MN, Alderson KL, Weiss JM, Ames E, Bruhn KW, Craft N, Wiltrout RH, Longo DL, Lanier LL, Blazar BR, Redelman D, Murphy WJ. Delineation of antigen-specific and antigen-nonspecific CD8(+) memory T-cell responses after cytokine-based cancer immunotherapy. Blood. 2012 Mar 29;119(13):3073-83. doi: 10.1182/blood-2011-07-369736