Cytomegalovirus infection of brain tumors and CMV immunotherapy

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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
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 immnosuppression 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 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 phosphoinositide-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
their pluripotent properties, the possibility of their transformation into tumor stem cells containing CMV cannot be 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.

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

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 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 carboptoxin 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 glioblastomas receive antiviral and anti-COX-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...
was found that a single intravenous injection of $3 \times 10^7$ cells 10-20-times with a significant increase in the level can stimulate the proliferation and CMV pP65-specific T-cells stimulated by autologous dendritic cells (DCs) preclinical study suggested that a patient's autologous 23 and 14 months [53]. Moreover, SK Nair 4 months, respectively) and median overall survival of after it, resulted in a 2.5-fold life prolongation (10 and 8.7 months, respectively) [49].

The stage of the disease affects the effectiveness of CMV immunotherapy with chemotherapy or radiation during immunotherapy, heterogeneity and low level of 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.
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. CMV 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.

Conclusion

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).

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