

CYTOMEGALOVIRUSES AND MALIGNANT BRAIN TUMORS

N.I. Lisyany, A.A. Klyuchnikova, L.N. Belskaya, A.A. Lisyany, I.A. Gnedkova*

SI "Institute of Neurosurgery, NAMS of Ukraine", Kyiv 04050, Ukraine

The review analyzes in detail the data on cytomegalovirus (CMV) as the cause of the development of malignant brain tumors. The use of modern methods of immunohistochemistry and polymerase chain reaction makes it possible to detect both individual proteins and CMV genes in tumor tissue, while virus cannot be isolated from tumor tissue using classical virological methods. The paper discusses the theories of "hit-and-run" and "microinfections", which explain the mechanism of action of CMV. The data on various molecular mechanisms of transformation of normal cells into tumor cells under the action of CMV are presented. The presence of CMV was shown not only in tumor cells, but also in neural stem cells, monocytes and macrophages. The possibility of using immunotherapy with T-lymphocytes and CMV-based dendritic cellular vaccines for the treatment of cancer patients is discussed in detail. Clinical data on their effectiveness are presented. Three possible mechanisms of the action of immunotherapeutic drugs containing CMV antigens are considered.

Key Words: cytomegalovirus (CMV), glioblastomas, stem cells, immunosuppression, immunotherapy, CMV containing preparations.

DOI: 10.32471/exp-oncology.2312-8852.vol-41-no-4.13899

Today, thanks to the use of new molecular genetic techniques and the improvement of the known immunohistochemical methods, a lot of data on the role of viral infection in oncogenesis and immunotherapy of malignant tumors have appeared. Taking part in a scientific discussion about viruses and cancer, More and Chang (2010) [1] argued that only 7 viruses are completely oncogenic and cause human tumors, which make up only 10–15% of all tumors known in the world. In principle, oncology, according to these authors, can be divided into infectious and non-infectious. Comparing the development of a viral infection with carcinogenesis, one can find many common characteristics, such as inflammation, innate immunity reactions, immunity suppression, etc. [1]. The role of many other viruses that are detected in tumor tissue, including cytomegalovirus (CMV), in tumor induction or oncostimulation has not yet been definitively proven, despite a large number of publications [1].

It is known that antibodies to CMV in the blood are detected in 80–85% of the adult population, and the infection itself is asymptomatic in healthy individuals. This virus is detected in tumor tissue in many cancers — in breast, intestinal, prostate, salivary gland cancer as well as in glioblastomas and medulloblastomas [2–9].

The presence of viral antigens in tumor parenchyma can be determined by immunohistological methods, but it is rarely possible to isolate CMV using classical virological methods, which led supporters of the CMV virus oncogenesis theory to propose "hit-and-run" hypothesis, which explained the frequent absence of the active virus in tumors and in the blood [10]. Somewhat later, the term "microinfection" of CMV in a tumor was also proposed to explain the conflicting data on the role of the virus in the induction

of tumors [11–13]. It was stated that the reason for the contradictory data lies in the imperfect research methods that were used by various authors. However, even the use of modern immunohistochemistry, gene-molecular methods such as polymerase chain reaction (PCR) did not always allow detection of CMV infection in tumors [14, 15].

Proponents of the CMV theory of neuro-oncogenesis give much evidence in its favor and point out the important role of CMV in the pathogenesis of the neoplastic process. Thus, it has been shown that US-28, a CMV-coded chemokine receptor, can bind chemokines of glial cells, which leads to their activation and stimulation of proliferation, the synthesis of pro-angiogenic factors, in particular, vascular endothelial growth factor. Injection of glial cells to mice expressing US-28 protein causes the development of tumors [16] and activates signal transducer and activator of transcription 3 — STAT-3 [17]. It was also shown that the conservative CMV proteins encoded by the 23/122 genes responsible for viral replication are able to induce the growth of glioblastomas [18, 19]. It has been shown that CMV infection induces phosphorylation of intracellular kinases, and IE-2 CMV protein binds to histone deacetylase-2, which enhances the transcriptional activity of cells [20]. At the same time, microRNA associated with CMV infection could be involved in the induction of tumors [21, 22].

Finally, CMV plays a role in the development of intratumoral immunosuppression, whether this suppression creates conditions for the development and maintenance of CMV in a tumor, or vice versa, CMV infection induces immunosuppression and thereby promotes tumor growth and suppression of antitumor immunity [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 STAT-3 transcription factor [24, 25], which is a key molecule in carcinogenesis and in immune suppression of the tumor [26, 27].

Submitted: June 25, 2019

*Correspondence: E-mail: nimun.neuro@gmail.com

Abbreviations used: CMV — cytomegalovirus; IL — interleukin;

NSC — neural stem cells; PCR — polymerase chain reaction.

As known, the STAT-3 factor is important for the activation and migration of neural stem cells (NSC) [28, 29]. In addition, CMV-induced IL-10 inhibits the synthesis of pro-inflammatory cytokines [30] and the proliferation of monocyte precursors [31]. This factor contributes to the conversion of proinflammatory monocytes M-1 into immunosuppressive M-2 monocytes, which suppress the functions of immune cells [32, 33]. It is believed that the presence of M-2 monocytes in the tumor is an unfavorable prognostic sign [33, 34].

According to the latest theories of oncogenesis, it is believed that glioblastomas develop from tumor stem cells [34, 35], which can occur as a result of disruption of normal differentiation of NSC into astrocytes under the influence of mutagenic factors or viruses [36]. Thus, it is known that brain NSCs express a platelet-derived growth factor receptor binding 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 involved in proliferation [36–38].

There are two stages of the interaction of CMV and tumor [3, 39]. At the first stage, CMV binds to the NCS via the platelet growth factor receptor and activates the STAT-3 factor, as stated above, which ensures the migration of these cells and the synthesis of IL-10. IL-10 acts on monocytes turning them into M-2 cells. The second stage of interaction of CMV and tumor is associated with M-2 cells, which accumulate in the tumor focus and indirectly stimulate tumor growth, affecting angiogenesis, the immune response, migration and invasion of tumor cells [39]. It is assumed that the interaction of CMV and host cells can lead to tumor induction or modulation of tumor growth, which can be realized, as noted above, by various mechanisms leading to inhibition or stimulation of one or another intracellular process [3, 39].

Thus, it is possible that the tumor microenvironment, namely, macrophage-monocytic cells and NSC may be the reservoir of the virus in the tumor, although there is an assumption that all or most of the tumor cells contain CMV proteins [40].

The theory of CMV involvement in the development of malignant brain tumors is not recognized by all researchers. In this regard, the study of 40 glial tumors, 31 meningiomas and 6 neurinomas for the presence of CMV or its antigens in tumors using PCR methods and immunohistochemistry is indicative [14]. In parallel, the authors investigated 76 blood samples to check for the presence of the virus in the blood and the possibility of its transition from blood to a tumor. PCR method could not be able to detect virus DNA both in tumors and in the blood of these patients. Immunohistochemistry also did not reveal the presence of CMV antigens. When studying the level of antibodies to CMV, as well as to Epstein — Barr virus, herpes zoster virus it was not possible to obtain significant differences in the level of antibodies in the general population. These results do not support the hypo-

thesis suggesting an association of herpes viruses and primary brain tumors [14].

Nevertheless, other studies confirm the presence of CMV proteins and genes in glial tumors, which are detected by immunohistochemical methods or PCR [38–40]. CMV can be present not only in cancer cells, but as noted above, in monocytes, normal and cancer stem cells, the number of tumor cells affected by CMV is very variable and depends largely on both the research method and the type of gene or proteins that were used [38, 40].

The detection of CMV and its antigens in glioblastoma cells served as the basis for the development of new approaches to virotherapy and immunotherapy of CMV-containing tumors. The very first studies were directed to the use of antiviral drugs alone or in combination with other inhibitors of proliferation or inflammation and immunotherapy [41–43]. However, despite the positive results in pilot studies in patients with glioblastomas, it was not possible to obtain a visible positive effect from specific antiviral therapy. A retrospective analysis of 50 patients with glioblastoma multiforme who received valganciclovir for 6 months together with standard treatment showed that the overall survival of patients was 25.0 months compared with 13.5 months in the control group [44, 45].

Nevertheless, on the basis of ideas about the different antigenicity of CMV-infected and non-infected tumor cells, new immunotherapy technologies were developed. It has been established that cytotoxic T-lymphocytes specific for CMV are able to lyse *ex vivo* CMV-infected glioblastoma cells [38], which served as the basis for using T-lymphocytes from blood of patients with CMV gliomas for autologous immunotherapy [41]. While T-lymphocytes of patients with gliomas were not sufficiently active, due to immunosuppression, their additional activation with IL-2 and CMV peptides led to an increase in cytotoxicity against tumor cells containing CMV antigens [41, 42]. These works drew the attention of researchers to two positions, namely that the patient's T-cells can be activated *ex vivo* with CMV antigens and cytokines and that the presence of CMV antigens in a tumor cell makes it susceptible to the action of lymphocytes and therefore such tumor cells can be target cells for cytotoxic lymphocytes [38, 41]. Unfortunately, the use of such T-cell adoptive therapy in the clinic did not give a significant positive result [41, 42], whereas the use of dendritic cells enriched with CMV antigens allowed to induce a sufficiently strong immune response, which led to the accumulation of cytotoxic lymphocytes in the tumor and lymph nodes [43]. The survival rate of patients who received vaccine based on dendritic cells associated with CMV increased to 36.5 months whereas in the control without vaccination was 18.5 months [43, 44].

The use of a dendritic cell vaccine enriched with CMV peptides in combination with T-cell therapy or temozolomide chemotherapy caused both CD-8 superactivation and prolonged survival of pa-

tients without tumor progression up to 25.3 months whereas in the control group without vaccination, the survival rate was 16.5 months [45–47]. It has been shown that the use of CMV dendritic cell vaccine against the background of chemotherapy with temozolomide leads to a stable specific immune response [48–50]. Several other variants of CMV dendritic and T-cell vaccines are being tested [44].

To explain the mechanism of action of different immune vaccines that are associated with CMV proteins and antigens, several theories have been proposed, among which the most popular are the direct, indirect, and cross-antigen-specific theory [44]. The essence of the direct theory is the destruction of CMV-positive cells by immune cells, including tumor stem cells, which contain much CMV. Immune cytotoxic and apoptotic destruction of glioblastoma cells containing CMV leads to a decrease in tumor size and lengthening the patient's life. The hypothesis of indirect immunotherapeutic action indicates that in addition to the direct effect on tumor cells in the tumor focus, the activity of macrophage and T-killers increases, as well as the synthesis of cytokines, in particular, interferons, which leads to the destruction of tumor cells that do not contain CMV [51, 52]. The cross-reaction hypothesis is that after the death of tumor cells, many tumor antigens are released which cause a second wave of activation of antitumor reactions that kill tumor cells regardless of their CMV content [44].

Summarizing the above results about the immunotherapeutic efficacy of CMV containing immune vaccines and technologies, it must be said that it is still early to draw definite conclusions. The results indicate only the existence of a large number of approaches to improve the specified method of immunotherapy of tumors. At a minimum, the effectiveness of immunotherapy of tumor cells directed against CMV can be realized by different mechanisms, both through specific anti-CMV immune responses and by cross-immune responses, which can lead to the destruction of both positive and negative CMV tumor cells.

At the same time, the proposal about the possibility of using antiviral CMV immunotherapy in the combined treatment of glioblastomas is not recognized by all researchers, and, according to Ranganathan *et al.* [40], such a recommendation is premature, and a few publications on this issue should be considered as preliminary and not convincing yet.

It is also known that CMV can persist in other human malignant tumors, not only in medulloblastomas and glioblastomas. This is probably the universal property of this virus to accompany any malignant processes in the body. In other words, we can talk only about the association of CMV with the malignancies, and tumors containing CMV are recommended to be called “CMV-associated glioblastomas or medulloblastomas” [40]. The practical use of this association for the development of new methods for the treatment of malignant brain tumors is still under

intensive study, and this gives us hope for obtaining new immunotherapeutic drugs.

REFERENCES

1. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer* 2010; **10**: 878–89.
2. Harkins LE, Matlaf LA, Soroceanu L, *et al.* Detection of human cytomegalovirus in normal and neoplastic breast epithelium. *Herpesviridae* 2010; **1**: 8–16.
3. Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? *Virus Res* 2011; **157**: 193–203.
4. Melnick M, Sedghizadeh PP, Allen CN. 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; **92**: 118–25.
5. Cobbs C, Harkins L, Samanta M, *et al.* Human cytomegalovirus infection and expression in human malignant glioma. *Cancer* 2002; **62**: 3347–50.
6. Scheurer ME, Bondy ML, Aldape KD, *et al.* Detection of human cytomegalovirus in different histological types of gliomas. *Acta Neuropathol* 2008; **116**: 79–86.
7. Geder L, Sanford EJ, Rohner EJ, *et al.* Cytomegalovirus and cancer of the prostate: in vitro transformation of human cells. *Cancer Treat Rep* 1977; **61**: 139–46.
8. Sanford EJ, Geder L, Laychock A, *et al.* Evidence for the association of cytomegalovirus with carcinoma of the prostate. *J Urol* 1977; **118**: 789–92.
9. Demmler GJ. What is CMV? 2010 [webpage] cited; Available from: www.congenitalcmv.org/public.htm
10. Shen Y, Zhu H, Shenk T. Human cytomegalovirus 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; **94**: 3341–5.
11. Cinatl JJr, Vogel JU, Kotchetkov R, *et al.* Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression. *FEMS Microbiol Rev* 2004; **28**: 59–77.
12. Samanta M, Harkin L, Klemm K, *et al.* High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. *J Urol* 2003; **170**: 998–1002.
13. Harkins L, Volk AL, Samanta M, *et al.* Specific localization of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet* 2002; **360**: 1557–63.
14. Poltermann S, Schlehofer B, Steindorf K, *et al.* Lack of association of herpesviruses with brain tumors. *J. Neurovirol* 2006; **12**: 90–9.
15. Lau S, Chen YY, Chen WG, *et al.* Lack of association of cytomegalovirus with human brain tumors. *Mod Pathol* 2005; **18**: 838–43.
16. Maussang D, Verzijl D, van Walsum M, *et al.* Human cytomegalovirus-encoded chemokine receptor US28 promotes tumorigenesis. *Proc Natl Acad Sci USA* 2006; **103**: 13068–73.
17. Slinger E, Maussang D, Schreiber A, *et al.* HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. *Sci Signal* 2010; **5**: 58–64.
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. *Gen Virol* 1998; **79**: 3039–47.
19. Cobbs CS, Soroceanu L, Denham S, *et al.* Modulation of oncogenic phenotype in human glioma cells by cyto-

megalovirus IE1-mediated mitogenicity. *Cancer Res* 2008; **68**: 724–30.

20. **Park JJ, Kim YE, Pham HT, et al.** Functional interaction of the human cytomegalovirus IE2 protein with histone deacetylase 2 in infected human fibroblasts. *J Gen Virol* 2007; **88**: 3214–23.

21. **Kluiser J, Kroesen B, Poppema S, et al.** The role of microRNAs in normal hematopoiesis and hematopoietic malignancies. *Leukemia* 2006; **20**: 1931–36.

22. **Huse JT, Brennan C, Hambardzumyan D, et al.** The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis *in vivo*. *Genes Dev* 2009; **23**: 1327–37.

23. **Popkin DL, Watson MA, Karaskov E, et al.** Murine cytomegalovirus paralyzes macrophages by blocking IFN gamma-induced promoter assembly. *Proc Natl Acad Sci USA* 2003; **100**: 14309–14.

24. **Wehinger J, Gouilleux F, Groner B, et al.** 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; **394**: 365–70.

25. **Spencer JV, Cadaoas J, Castillo PR, et al.** Stimulation of B lymphocytes by cmvIL-10 but not LAcmvIL-10. *Virology* 2008; **374**: 164–9.

26. **Abou-Ghazal M, Yang W, Qiao W, et al.** The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. *Clin Cancer Res* 2008; **14**: 8228–35.

27. **Brantley EC, Benveniste EN.** Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas. *Mol Cancer Res* 2008; **6**: 675–84.

28. **Sherry MM, Reeves A, Wu JW.** STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. *Stem Cells* 2009; **27**: 2383–92.

29. **Wei J, Barr J, Kong LY, et al.** Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. *Mol Cancer Ther* 2006; **9**: 67–8.

30. **Spencer JV, Lockridge KM, Barry PA, et al.** Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol* 2002; **76**: 1285–92.

31. **Mildner A, Schmidt H, Nitsche M, et al.** Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* 2007; **10**: 1544–53.

32. **O'Farrell AM, Liu Y, Moore KW, et al.** IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: Evidence for Stat3-dependent and independent pathways. *EMBO J* 1998; **17**: 1006–18.

33. **Lin E, Li JF, Gnatovskiy L, et al.** Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; **66**: 11238–46.

34. **Alcantara Llaguno S, Chen J, Kwon CH, et al.** Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell* 2009; **15**: 45–56.

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; **82**: 9994–10007.

36. **Odeberg J, Wolmer N, Falci S, et al.** Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes. *J Neurosci Res* 2007; **85**: 583–93.

37. **Soroceanu L, Akhavan A, Cobbs C.** Platelet-derived growth factor- α receptor activation is required for human cytomegalovirus infection. *Nature* 2008; **455**: 391–5.

38. **Lucas KG, Bao L, Bruggeman R, et al.** The detection of CMV pp65 and IE1 in glioblastoma multiforme. *J Neurooncol* 2011; **103**: 231–8.

39. **Soderberg-Naucler C.** HCMV microinfections in inflammatory diseases and cancer. *J Clin Virol* 2008; **41**: 218–23.

40. **Ranganathan PA, Clark P, Kuo J, et al.** Significant association of multiple human cytomegalovirus genomic loci with glioblastoma multiforme samples. *J Virol* 2012; **86**: 854–64.

41. **Schuessler A, Smith C, Beagley L, et al.** Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Res* 2014; **74**: 3466–76.

42. **Ghazi A, Ashoori A, Hanley PJ, et al.** Generation of polyclonal CMV-specific T cells for the adoptive immunotherapy of glioblastoma. *J Immunother (Hagerstown, Md: 1997)* 2012; **35**: 159–168.

43. **Rahbar A, Stragliotto G, Orrego A, et al.** Low levels of human cytomegalovirus infection in glioblastoma multiforme associates with patient survival: A case-control study. *Herpesviridae* 2012; **3**: 3.

44. **Rahman M, Dastmalchi E, Karachi A, Mitchel D.** The role of CMV in glioblastoma and implications for immunotherapeutic strategies. *Oncoimmunology* 2019; **8**: e1514921.

45. **Peng C, Wang J, Tanksley JP, et al.** Valganciclovir and bevacizumab for recurrent glioblastoma: A single-institution experience. *Mol Clin Oncol* 2016; **4**: 154–8.

46. **Reap EA, Suryadevara CM, Batich KA, et al.** Dendritic cells enhance polyfunctionality of adoptively transferred T cells that target cytomegalovirus in glioblastoma. *Cancer Res* 2018; **78**: 256–64.

47. **Reap EA, Suryadevara CM, Batich KA, et al.** Long-term survival in glioblastoma with cytomegalovirus pp65-targeted vaccination. *Clin Cancer Res* 2017; **23**: 1898–1909.

48. **Asavaroengchai W, Kotera Y, Mule JJ.** Tumor lysate-pulsed dendritic cells can elicit an effective antitumor immune response during early lymphoid recovery. *Proc Natl Acad Sci USA* 2002; **99**: 931–6.

49. **Sanchez-Perez L, Choi BD, Reap EA, et al.** BLyS levels correlate with vaccine-induced antibody titers in patients with glioblastoma lymphodepleted by therapeutic temozolomide. *Cancer Immunol Immunother* 2013; **62**: 983–7.

50. **Prins RM, Cloughesy TF, Liau LM.** Induction of cytomegalovirus-specific anti-tumor immunity after autologous tumor lysate-pulsed dendritic cell vaccination in a patient with glioblastoma. *New England J Med* 2008; **359**: 539–41.

51. **Tietze JK, Wilkins DE, Sckisel GD, et al.** Delineation of antigen-specific and antigen-nonspecific CD8(+) memory T-cell responses after cytokine-based cancer immunotherapy. *Blood* 2012; **119**: 3073–83.

52. **Paijo J, Doring M, Spanier J, et al.** GAS senses human cytomegalovirus and induces type I interferon responses in human monocyte-derived cells. *PLoS Pathogens* 2016; **12**: e1005546.