Journal of NeuroVirology
https://doi.org/10.1007/s13365-020-00901-9

CASE REPORT

Human cytomegalovirus DNA detection in a recurrent glioblastoma multiforme tumour, but not in whole blood: a case report and discussion about the HCMV latency and therapy perspectives

Emiliya Nikolova[1,2] · Petia Dimova[3] · Krasimir Minkin[4] · Tihomir Todorov[2] · Vanio Mitev[1] · Albena Todorova[1,2]

Received: 17 May 2020 / Revised: 1 August 2020 / Accepted: 21 August 2020

Abstract

In the current study, a 58-year-old male patient presented with recurrent glioblastoma multiforme (GBM). The patient underwent surgical resection, 4 months earlier, followed by radiotherapy and chemotherapy. During the second surgical intervention, tumour tissue and whole blood were sampled and analysed for human cytomegalovirus (HCMV) DNA, immediate early (IE) mRNA and pp65 mRNA. HCMV DNA was detected only in the recurrent tumour tissue but not in the whole blood. Neither IE mRNA nor pp65 mRNA was expressed. Our result suggests HCMV latency in the brain tumour with detectable level of viral DNA. More data are needed to understand the HCMV infection chronology in the brain tumours but our data could be important for further studies of HCMV antigens on the tumour surface and anti-GBM therapy.

Keywords Human cytomegalovirus · Glioblastoma multiforme · Anti-tumour therapy

Introduction

In the past decades, the role of HCMV infection in the development and progression of brain tumours became a source of scientific research and debate. Even during the latency, HCMV is able to modify the oncogenic phenotype of the cell, through the so-called mechanism of oncomodulation by disturbing critical signalling pathways (Cinatl et al. 1996). Primary and secondary brain tumours have been found positive for HCMV proteins and nucleic acids, but adjacent non-tumoral brain tissue was negative (Cobbs et al. 2002; Mitchell et al. 2008). Moreover, GBM patients with low viral blood load have longer OS time compared with patients with high viral blood load (Rahbar et al. 2013).

The aim of our study is to present a case report of Bulgarian patient with recurrent GBM and detection of HCMV DNA only in the tumour tissue but not in whole blood. Additionally, the analysis of viral immediate early (IE) protein mRNA and pp65 mRNA showed lack of expression. Our result suggests HCMV latency in the brain tumour with detectable level of viral DNA. There are not enough data of HCMV latency in the brain tumours but our data could be important for further analyses of HCMV antigens on the tumour cell surface and the development of targeted anti-HCMV and anti-GBM therapy.

Case report

A 58-year-old man was diagnosed with GBM. First surgical operation was 4 months earlier, after 1-month clinical history of left-sided hemiparesis and right-sided headache, with brief focal seizures of probable right-sided insular origin. Brain magnetic resonance imaging (MRI) revealed glial tumour in the right insula (Fig. 1a). Complete tumour resection using intraoperative neuromonitoring was performed (Fig. 1b). Histopathological examination identified GBM (WHO grade IV). Approximately 20 days after the operation, the patient was referred for chemo- and radiotherapy. Postoperative

✉ Emiliya Nikolova
nikolova.emily@gmail.com

[1] Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University – Sofia, Sofia, Bulgaria

[2] Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria

[3] Epilepsy Center, Department of Neurosurgery, University Hospital 'St. Ivan Rilski', Sofia, Bulgaria

[4] Department of Neurosurgery, University Hospital 'St. Ivan Rilski', Sofia, Bulgaria

Published online: 03 September 2020

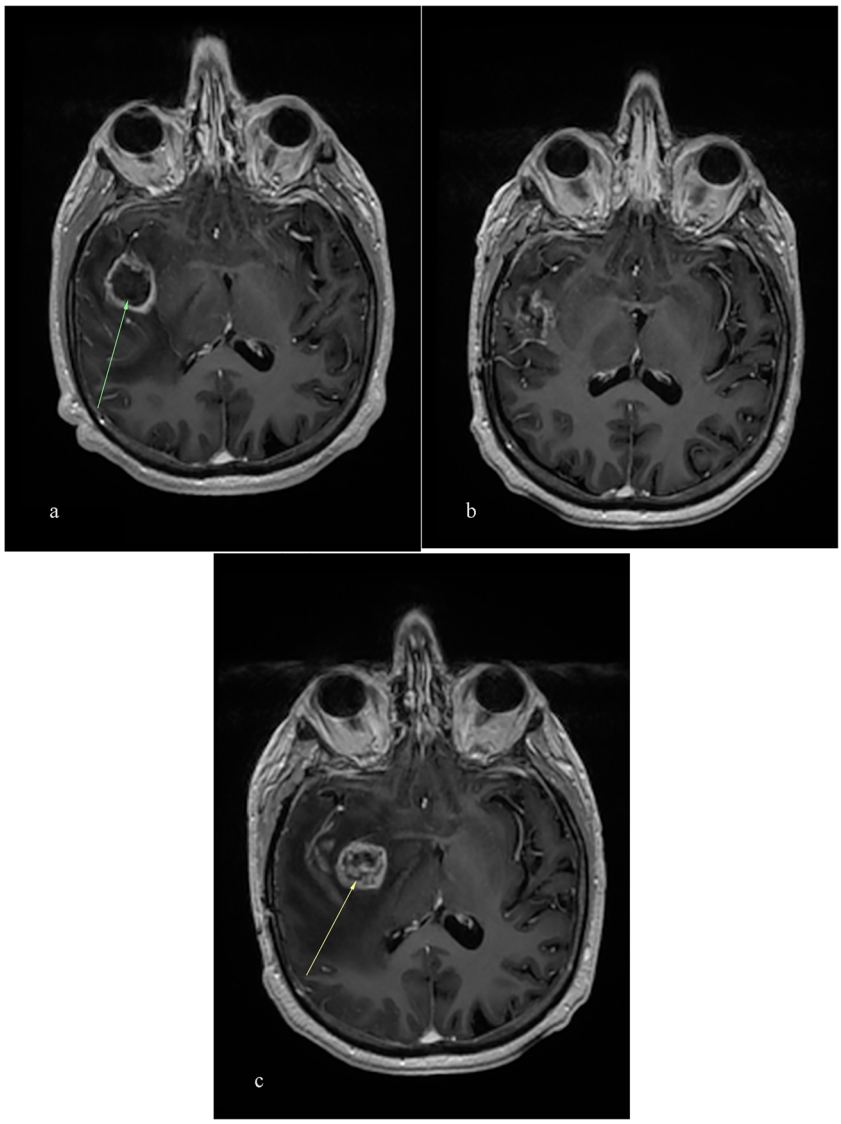

Fig. 1 **a** Preoperative MRI visualization of GBM. **b** Postoperative MRI, after complete resection. **c** Recurrence of GBM medial to the previous location

medical treatment included dexamethasone 8 mg/day and levetiracetam 1000 mg/day.

Four months later, the patient was readmitted for somnolence, worsening of the hemiparesis, dysarthria and locomotor ataxia. Brain MRI showed GBM recurrence in the right internal capsule and basal ganglia (Fig. 1c).

Blood and biochemical analyses were normal. Reoperation under intraoperative neuromonitoring with subtotal tumour resection because of basal ganglia involvement was performed. Postoperative medical treatment was not changed. The patient succumbed 6 months later.

The patient provided a written informed consent and the Ethical Committee of Medical University Sofia approved the study. During the second surgical resection, whole blood and tumour tissue were collected. DNA was extracted using phenol:chloroform:isoamyl alcohol (25:24:1). Proteinase K (100 μg/mL) was used for digestion. DNA was precipitated with 3 M ammonium acetate and ethanol. Pellets were resuspended in 100 μL of TE buffer and stored at − 20 °C. AmpliSens® CMV–FEP PCR kit with specific primers for targeted detection of HCMV *Pol* gene was used and the reaction was carried out according to the manufacturer's instructions (AmpliSens, Russia).

In order to analysed HCMV reactivation, we analysed viral tegument protein—pp65 and immediate early (IE) mRNAs. Total RNA was extracted using RNeasy Mini Kit (Qiagen). DNase I treatment was performed of the eluted total RNA. Reverse transcription was carried out using the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Primer sequences for detection of pp65 and IE cDNA are as follows: 5′–GTACCTGGAGTCCTTCTGCG-3′, 5′–CATCCAGC ATGATGTGCGAG-3′, 5′–CTCTGTCCTCAGTAATTGTG GCTG-3′ and 5′–GCAACTTCCTCTATCTCAGACACTG-3′. Primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene with the following sequence: 5′-ACCACA

GTCCATGCCATCAC-3′ and 5′-TCCACCCTGTTGCT GTA-3′ were used as an internal control (Kala et al. 2015). Positive and negative controls were included for mRNA expression analyses. A sample with an active HCMV infection was used as a positive control after obtaining a written informed consent from the patient.

PCR was conducted using 10 μL cDNA, 10 μM of each primer and 5x HOT FIREPol "EvaGreen" qPCR Supermix (Solis BioDyne) according to the manufacturer's instructions. The PCR conditions were as follows: 95 °C for 12 min, 35 cycles of 95 °C for 15 s and 60 °C for 30 s. All the PCR reactions were performed on real-time PCR—DTPrime (DNA Technology, Russia). Additionally, information about the genetic aberrations detected in the tumour tissue was provided.

Discussion

More than 15% of cancers are due to infectious agents. The untreated inflammation has the potential to generate reactive oxygen species (ROS) and trigger neoplastic transformation (Plummer et al. 2016). There are lots of controversial reports regarding HCMV related to brain tumours. High rate of HCMV DNA in peripheral blood has been observed in 80% of the tested patients (Mitchell et al. 2008). Contrary, Lehrer et al. (2015) have not found HCMV DNA in their group of 14 patients. It is clear that the suppressed immune microenvironment, around the GBM, is a perfect condition for reactivation of the latent HCMV (See et al. 2015).

In the Bulgarian patient, HCMV DNA was positive in the recurrent GBM tumour tissue but negative in whole blood. Additionally, we analysed the expression of mRNA of pp65 (tegument protein with immunomodulatory role) and IE (one of the first transcribed genes during HCMV lytic infection) but did not find any expression of these two genes. Our data suggests that there was little or no virus replication, typical for HCMV latent state. During the virus latency, different genes like US28 and LUNA (latency unique nuclear antigen) are expressed and their products regulate the cell signalling pathways and can trigger the host immunity (Duan et al. 2014). Schneider et al. (2017) have reported positive serum anti-HCMV IgM and IgG in patients with high-grade gliomas, but they have not detected any viral DNA in peripheral blood of the IgM positive patients. Probably, anti-HCMV IgM was triggered by latency-related HCMV genes or by locally reactivated brain tumour infection.

The lack of mRNA expression might also be due to the following:

- The sample age—the sample was collected 2 years before the mRNA analysis and mRNA degradation is possible.
- Low sensitivity of these markers—there are data that mRNA of IE is less sensitive markers in the late phase of HCMV infection (Revello et al. 2001).
- Heterogenic distribution of HCMV in brain tumour cells—although part from one tumour, different tumour cells used for DNA and mRNA isolation and analyses may content HCMV infection in different phases.

Probably HCMV is not distributed to all GBM cells and single-cell analyses might elucidate the chronology of HCMV latency, reactivation and gene expression. HCMV has affinity to GBM stem cells and the process of oncomodulation further increases GBM malignancy and stemness (Soroceanu et al. 2015). It is known that the higher levels of HCMV virions are associated with poorer prognosis in GBM patients (Rahbar et al. 2013).

The genetic profiling of the analysed tumour tissue showed negative prognostic factors like the lack of MGMT promoter methylation, the EGFR amplification, the IDH-1/2-wild type and the CDKN2A deletion are related to a more aggressive tumour nature (Table 1).

The current treatment for GBM is the maximal and safe surgical resection, followed by radiation therapy and chemotherapy (Orringer et al. 2012). Nevertheless, GBM is still "hard-to-treat" tumour with low long-term survival and high rate of tumour recurrence. Novel more effective and specific anti-GBM therapies are in huge demand. Interestingly, HCMV has a high affinity for GBM stem cells that are in the basis of GBM resistance and recurrence (Soroceanu et al. 2015). The subventricular zone, reach in GBM stem cells, is a place where the mouse cytomegalovirus frequently reactivates from latency (Tsutsui et al. 2002). Some of the anti-HCMV drugs like cidofovir (Hadaczek et al. 2013) and valganciclovir (Stragliotto et al. 2013) have shown some positive effects on GBM. Recent clinical trials tested autologous dendritic cells targeting HCMV protein—pp65 and cytotoxic CMV-specific T cells in combination with standard anti-GBM therapy, and reported increased OS to 41.1 and 79.8 months respectively. Furthermore, few long-term survivors have been

Table 1 Genetic aberrations detected in the tumour tissue

Gene	Status
IDH 1/2	Normal (wild type)
MGMT promoter methylation	Negative
EGFR	Amplification
CDKN2A	Deletion
TP53	Normal (wild type)

IDH isocitrate dehydrogenase; *MGMT* O^6 -methylguanine-DNA methyltransferase; *EGFR* epidermal growth factor receptor; *CDKN2A* cyclin-dependent kinase inhibitor 2A; *TP53* tumour protein p53

reported (Batich et al. 2017; Schuessler et al. 2014). The emerging amount of scientific data on HCMV in brain cancer patients indicates the usefulness of HCMV status monitoring.

There are not enough data on the HCMV chronology of latency and reactivation in human GBM. Probably HCMV latency state could explain the better therapeutic effect of anti-HCMV immunotherapy targeting different viral proteins than anti-HCMV medications like cidofovir and valganciclovir inhibiting mainly viral DNA synthesis (Hadaczek et al. 2013; Stragliotto et al. 2013).

In conclusion, in our patient, HCMV DNA was detected only in the recurrent tumour tissue but not in whole blood. Additionally, neither IE mRNA nor pp65 mRNA was expressed in the analysed sample, suggesting a latent infection. More data are needed to understand the mechanisms and chronology of HCMV latency and reactivation in GBM, its potential to trigger a HCMV-specific immune response and the development of anti-HCMV immunotherapy for GBM treatment.

Acknowledgements We thank Teodora Tasseva from Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences, Slavena Atemin and Zornitsa Pavlova from Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria.

Author's contributions All authors approved the contents of the manuscript and validated the accuracy of the data.

Funding This work was supported in part by Medical University – Sofia, grants D-118/2018 and D-127/2019.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Batich KA, Reap EA, Archer GE, Sanchez-Perez L, Nair SK, Schmittling RJ, Norberg P, Xie W, Herndon JE 2nd, Healy P, McLendon RE, Friedman AH, Friedman HS, Bigner D, Vlahovic G, Mitchell DA, Sampson JH (2017) Long-term survival in glioblastoma with cytomegalovirus pp65-targeted vaccination. Clin Cancer Research 23: 1898–1909. https://doi.org/10.1158/1078-0432.CCR-16-2057

Cinatl J Jr, Cinatl J, Vogel JU, Rabenau H, Kornhuber B, Doerr HW (1996) Modulatory effects of human cytomegalovirus infection on malignant properties of cancer cells. Intervirology 39:259–269. https://doi.org/10.1159/000150527

Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG, Britt WJ (2002) Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res 62:3347–3350

Duan YL, Ye HQ, Zavala AG, Yang C, Miao L, Fu B, Seo K, Davrinche C, Luo M, Fortunato E (2014) Maintenance of large numbers of virus genomes in human cytomegalovirus-infected T98G glioblastoma cells. J Virol 88(7):3861–3873. https://doi.org/10.1128/JVI.01166-13

Hadaczek P, Ozawa T, Soroceanu L, Yoshida Y, Matlaf L, Singer E, Fiallos E, James CD, Cobbs CS (2013) Cidofovir: a novel antitumor agent for glioblastoma. Clin Cancer Res 19:6473–6483. https://doi.org/10.1158/1078-0432.CCR-13-1121

Kala R, Shah HN, Martin SL, Tollefsbol TO (2015) Epigenetic-based combinatorial resveratrol and pterostilbene alters DNA damage response by affecting SIRT1 and DNMT enzyme expression, including SIRT1-dependent γ-H2AX and telomerase regulation in triple-negative breast cancer. BMC Cancer 15:672. https://doi.org/10.1186/s12885-015-1693-z

Lehrer S, Green S, Rosenzweig KE, Rendo A (2015) No circulating human cytomegalovirus in 14 cases of glioblastoma. Neuro-Oncology 17:320. https://doi.org/10.1093/neuonc/nou325

Mitchell DA, Xie W, Schmittling R, Learn C, Friedman A, McLendon RE, Sampson JH (2008) Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. Neuro-Oncology 10:10–18. https://doi.org/10.1215/15228517-2007-035

Orringer D, Lau D, Khatri S, Zamora-Berridi GJ, Zhang K, Wu C, Chaudhary N, Sagher O (2012) Extent of resection in patients with glioblastoma: limiting factors, perception of resectability, and effect on survival. J Neurosurg 117:851–859. https://doi.org/10.3171/2012.8.JNS12234

Plummer M, de Martel C, Vignat J (2016) Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Heal 4:609–616. https://doi.org/10.1016/S2214-109X(16)30143-7

Rahbar A, Orrego A, Peredo I, Dzabic M, Wolmer-Solberg N, Straat K, Stragliotto G, Soderberg-Naucler C (2013) Human cytomegalovirus infection levels in glioblastoma multiforme are of prognostic value for survival. J Clin Virol 57:36–42. https://doi.org/10.1016/j.jcv.2012.12.018

Revello MG, Lilleri D, Zavattoni M, Stronati M, Bollani L, Middeldorp J, Gerna G (2001) Human cytomegalovirus immediate-early messenger RNA in blood of pregnant women with primary infection and of congenitally infected newborns. The Journal of Infectious Diseases 184:1078–1081. https://doi.org/10.1086/323425

Schneider M, Reitter EM, Kastner MT, Thannesberger J, Rieder F, Preusser M, Marosi C, Steininger C (2017) Absence of CMV viremia in high-grade glioma patients under low dose glucocorticoid treatment. Neuro-Oncology 19:1280–1282. https://doi.org/10.1093/neuonc/nox065

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 (2014) Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. Cancer Res 74:3466–3476. https://doi.org/10.1158/0008-5472.CAN-14-0296

See AP, Parker JJ, Waziri A (2015) The role of regulatory T cells and microglia in glioblastoma-associated immunosuppression. J Neuro-Oncol 123:405–412. https://doi.org/10.1007/s11060-015-1849-3

Soroceanu L, Matlaf L, Khan S, Akhavan A, Singer E, Bezrookove V, Decker S, Ghanny S, Hadaczek P, Bengtsson H, Ohlfest J, Luciani-Torres MG, Harkins L, Perry A, Guo H, Soteropoulos P, Cobbs CS (2015) Cytomegalovirus immediate-early proteins promote stemness properties in glioblastoma. Cancer Res 75:3065–3076. https://doi.org/10.1158/0008-5472.CAN-14-3307

Stragliotto G, Rahbar A, Solberg NW, Lilja A, Taher C, Orrego A, Bjurman B, Tammik C, Skarman P, Peredo I, Söderberg-Nauclér C (2013) Effects of valganciclovir as an add-on therapy in patients with cytomegalovirus-positive glioblastoma: a randomized, double-blind, hypothesis-generating study. Int J Cancer 133:1204–1213. https://doi.org/10.1002/ijc.28111

Tsutsui Y, Kawasaki H, Kosugi I (2002) Reactivation of latent cytomegalovirus infection in mouse brain cells detected after transfer to brain slice cultures. J Virol 76(14):7247–7254