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Non-Detection of HCMV Total Genomic DNA in Human Glioma Cells Genome

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ABSTRACT

AIM: To demonstrate if the human cytomegalovirus (HCMV) genome, that is involved in the pathogenesis of gliomas, is part of the genomic DNA of glioma cells or not.

MATERIAL and METHODS: The study included U87MG glioblastoma cell culture and tumor samples from glioma patients. The genomic DNA of tumor samples and U87MG cells were extracted and real-time quantitative PCR was used to assess the presence of the human cytomegalovirus genomic DNA.

RESULTS: Consequently, HCMV positivity was not detected in the tumor and cell line genomic DNA under the aforementioned experimental conditions.

CONCLUSION: We found that the genomic DNA of all the samples was negative for HCMV genomic DNA. Thus, HCMV could not be detected in human glioma tumors and we put forward that HCMV genomic DNA was not incorporated into the genomic DNA of glioma cells. Thus, total viral DNA is not involved in the pathogenesis of glioma; however, small viral particles or specific genes might be incorporated into the genomic DNA of glioma cells, leading to cancer development. This prompts further studies for verification.

KEYWORDS: Glioma, Surgical treatment, HCMV, qPCR

INTRODUCTION

Gliomas are the most prevalent tumors of the human central nervous system (CNS). Astrocytoma (a type of glioma) cells are star-shaped and arise from astrocytes (12). Astrocytoma cells are star-shaped and arise from astrocytes. They are generally graded using a scale of I to IV, describing their degree of malignancy. Grade IV astrocytoma is called Glioblastoma (GBM), which is divided into two types according to the World Health Organization (WHO) 2016 CNS malignancy classification (8). The first type is glioblastoma isocitrate dehydrogenase (IDH)-wildtype (about 90% of patients); it is described as primary or *de novo* glioblastoma. The second type is the glioblastoma IDH-mutant type (about 10% patients), also called secondary glioblastoma (8,11,12,15). GBM accounts for 15% of all brain tumors, often in adults of age 45–70 years. Even though the average age at which GBM develops is 53 years, approximately 8.8% of cases occur in children and the symptoms revealed by the disease are unspecific. This explains why the disease is often detected at advanced stages. The treatment of patients with glioblastoma includes surgical excision, chemotherapy, and adjuvant radiation therapy. The prognosis for GBM is poor, and the mean survival is less than 15 months (15,26). Many defects lead to the development of glioblastoma, for example, mutation of the p53 protein (a tumor suppressor) and overexpression of the proto-oncogene platelet-derived growth factor receptor is involved in the pathogenesis of the disease (4). In addition, human cytomegalovirus (HCMV) proteins and oligonucleotides are highly expressed in gliomas (2).

HCMV, a double-stranded DNA virus, is nearly 230 kb and belongs to the beta herpesvirus family (1,18). A significant portion of the world's population (especially adults in devel-

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oping countries) is infected with HCMV. This infection is not lethal under normal conditions, but it can be life-threatening in newborns and immunocompromised patients (6,21–23,28); however, HCMV is not considered an oncovirus (3,9,13,24) numerous recent investigations have suggested that HCMV might be involved to a wide range of disorders, including circulatory system disorders and various cancers (such as gliomas) (10,16,18).

In this study, we assessed the involvement of HCMV genomic DNA in glial tumor cells and determined the extensiveness of HCMV infection in patients with high-grade glial tumors in the Çukurova Region of Turkey.

MATERIAL and METHODS

Sources of Materials

The glioma tumor samples were obtained from the patient group of 6 men and 4 women of 19–74 years age range (mean 55.7, median 61). Before inclusion, informed consent was obtained from all the participants. The histopathological investigations were performed in the medical pathology laboratory.

U87MG glioblastoma cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, Lonza, Belgium), with 10% fetal bovine serum (Biowest, South America) added as a supplement.

Table I: Real-Time PCR Conditions

Hot start enzyme activation: 95°C 10 min.		
Denaturation: 95°C 15 sec Annealing: 65°C 30 sec Extention: 72°C 20 sec	]	45 cycles

Storage of Samples

Tumor samples were stored at –80°C before analysis.

Ethical Approval

The study was approved by Clinical Research Ethics Committee of Çukurova University (Date: July 18, 2014; No: 33/18).

Genomic DNA Isolation

Genomic DNA was extracted from frozen tumor sections and culture cells using a Quick-gDNA™ MiniPrep DNA isolation kit (Zymo Cat No: D3025), as per the manufacturer's instructions.

Real-time qPCR

Fifty nanograms of extracted genomic DNA were added to each qPCR reaction. Sample and control DNAs were amplified by 45 cycles with the conditions described in Table I, using the Artus CMV RG PCR Kit (Qiagen-Cat No: 4503203) and the Qiagen Rotor-Gene Real-Time PCR system.

RESULTS

We extracted DNA from tumor samples and glioblastoma cell culture and the concentrations were measured using Qubit 3.0 Fluorimeter (Thermofisher). The reaction amplification curves and colors of the samples are illustrated in Figure 1. We performed real-time PCR experiments using tumor DNA and 1000000, 100000, 10000, and 1000 copies of HCMV-positive control DNA. Table II displays the clinical details of the patients and the outcomes of the qPCR test for HCMV DNA. Consequently, HCMV positivity was not detected in the tumor genomic DNA under the aforementioned experimental conditions.

DISCUSSION

Previous studies have revealed a relationship between the presence of HCMV DNA, RNA, protein particles, and the progression and development of glioma. Some of the HCMV

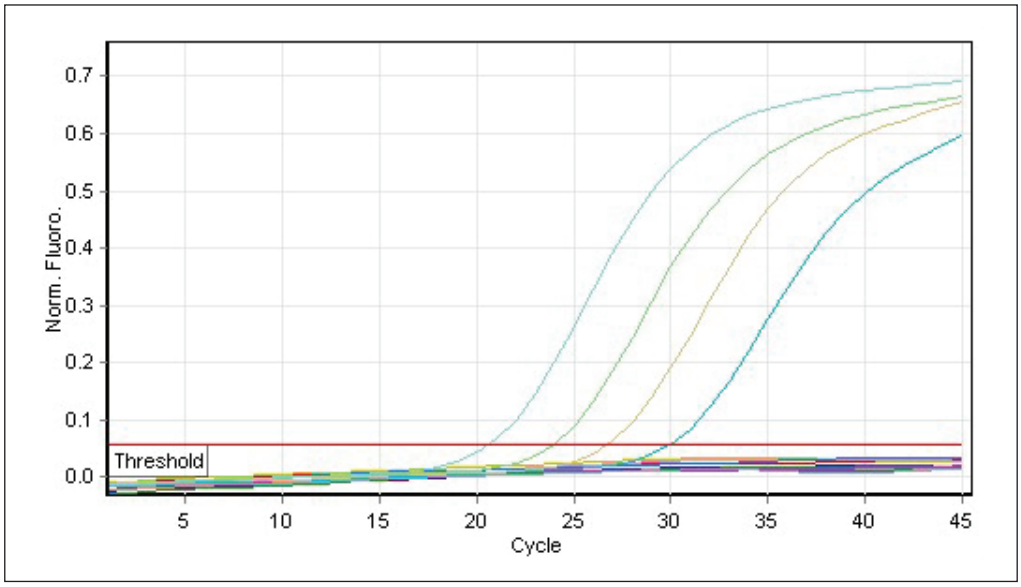

Figure 1: Amplification curve of real time PCR assay for the DNA isolated from tumor tissues, glioblastoma cell culture and control HCMV + DNA.

Table II: Samples of Real-Time PCR Reaction, Quantitation and Histological Data

No.	Color	Name	Type	Ct	Given Conc (copies/ml)	Calc Conc (copies/ml)	Age	Sex	Location of tumor	Histology/ disease
1		1	Tumor DNA	-	-	-	60	Female	Left Frontal Location	Glioblastoma
2		2	Tumor DNA	-	-	-	71	Male	Temporoparietal Location	Glioblastoma
3		3	Tumor DNA	-	-	-	74	Male	Right Temporal Region	Glioblastoma
4		4	Tumor DNA	-	-	-	56	Female	Temporoparietal Location	Glioblastoma
5		5	Tumor DNA	-	-	-	71	Male	Right Temporal Region	Glioblastoma
6		8	Tumor DNA	-	-	-	47	Male	Right Temporal Region	Glioblastoma
7		9	Tumor DNA	-	-	-	53	Male	Temporal Lobe	Glioblastoma
8		11	Tumor DNA	-	-	-	69	Male	Left frontoparietotemporal	Glioblastoma
9		12	Tumor DNA	-	-	-	40	Female	Frontal Location	Glioblastoma
10		14	Tumor DNA	-	-	-	47	Female	Frontal Location	Glioblastoma
12		sT1	Standard	30.05	1,000	981				
13		sT2	Standard	26.83	10,000	10,907				
14		sT3	Standard	24.02	100,000	89,143				
15		sT4	Standard	20.73	1,000,000	1,048,837				
16		NK	Negative Control	-	-	-				

No: *Number,* ***Ct:*** *Threshold cycle,* ***Conc:*** *concentration,* ***Calc:*** *Calculated. Each color corresponding to the different amplification curves shown in Figure 1 is provided with its sample and standard in Table II.*

particles have been found to be present in glial tumors and it has been suggested that these viral particles have oncogenic properties in glioma cells.

In an early study, the existence of HCMV proteins and DNA in glioblastoma samples was analyzed and as a result, IE1, pp65, pp28, US28, gB, HCMV IL-10, IE1, and gB HCMV gene particles and proteins were identified in cancer tissues. Normal brain tissues, surrounding the tumor, had no HCMV protein or nucleotide (5). Immunohistochemistry studies revealed that HCMV immediate early 1 (IE1) protein is present in 100% of glioblastomas and 82% of low-grade gliomas, according to Scheurer et al. Moreover, HCMV-specific oligonucleotides were detected using *in situ* hybridization (ISH) (14). Another study revealed the presence of human cytomegalovirus DNA, RNA, and protein in varying degrees of fifty-two glioma cases in Brazil. Using qPCR, *in situ* hybridization (ISH), and immunohistochemistry (IHC), the UL83 virus region, the early beta 2.7 RNA, and protein was found in 73%, 36%, and 57% of patients with gliomas, respectively. The presence of the virus particles was independent of the tumor type or grade. This depicts that there is no correlation between virus infection and tumor development (19). An intriguing study looked at the expression of HCMV genes and the neovascularization marker endocan in 79 brain samples from glioma patients and 8 brain samples from healthy controls. In glioma samples, HCMV pp65 protein and DNA were found in 52 (65.8%) and 43 (54.4%) out of 79 samples, respectively (27). Few studies have shown that some antiviral medications, especially effective for HCMV infections, are also promising for the treatment of glioma (2,17,20). These effects could be linked to the presence of HCMV particles. Perhaps, based on the potential role of HCMV in glioma, the virus antigens are the main target of combinatorial therapy. In a randomized, double-blind study in Sweden, the antiviral agent valganciclovir (valcyte) was added to the standard chemotherapy for glioblastoma and preliminary results were hopeful. Recently, the CMV antigens have become the prime focus for immunotherapeutic strategies. In clinical studies, autologous dendritic cells are pulsed with CMV antigens to activate a cellular immune response, CMV-specific autologous T-cells were selected and reintroduced to the patient, and CMV peptides were used for autologous vaccination (25). Building on aforementioned studies, we sought to determine whether the cellular genomic DNA in the glioma tumor samples included any HCMV genomic DNA in the Çukurova Region of Turkey. According to our real-time PCR assay results, HCMV genomic DNA was undetectable in the tumor tissue DNA. The results are coherent with other studies

that included glioblastoma patients and real-time quantitative PCR (qPCR) did not detect HCMV DNA in the tumor cells from any sample (29); however, our results do not correlate with the previous reports, indicating that HCMV DNA was detectable in some fractions of human glioblastoma samples. The possible reason for the detection of HCMV particles in the glioma samples of early studies could be explained by the fact that glioblastoma is often accompanied by necrosis of microglial cells that originate from bone marrow myeloid cells; these cells might be HCMV-infected. Another justification could be that the samples were contaminated or HCMV DNA was already present in the histology lab (7). On the contrary, it was considered that small HCMV genomic particles or specific genes (not targeted by the qPCR reaction in our study) could be incorporated into the genomic DNA of glioma cells, leading to cancer development.

CONCLUSION

In conclusion, we found that HCMV genomic DNA was negative in all samples. Thus, it can be put forward that HCMV genomic DNA is not included in the genomic DNA of glioma cells. However, small viral particles or specific genes may be incorporated into the genomic DNA of glioma cells, leading to cancer development. This prompts further research for validation.

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AUTHORSHIP CONTRIBUTION

Study conception and design: DG
Data collection: DMY, DG
Analysis and interpretation of results: MDOA, DG
Draft manuscript preparation: MDOA, DG
Critical revision of the article: DG, MDOA, DMY
Other (study supervision, fundings, materials, etc...): DG
All authors (DG, MDOA, DMY) reviewed the results and approved the final version of the manuscript.

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