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Human Cytomegalovirus (HCMV) infection was not correlated with overall survival in glioblastomas

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There are many arguments about the presence of HCMV (Human Cytomegalovirus) in malignant gliomas. This study was to investigate the presence and prognostic value of HCMV in glioblastomas. 68 patients including 64 primary glioblastomas and 4 secondary glioblastomas were involved in this study. Immunofluorescence was adopted for detecting glycoprotein B (gB) and glycoprotein H (gH) of HCMV in glioblastoma tissues. Kaplan–Meier Analysis and Chi Square were used to evaluate patients' survival and the association between HCMV infection and patients' characteristics, respectively. We found that the presence rate of gB and gH in glioblastomas were 48.5% (33/68) and 42.6% (29/68), respectively. The co-occurrence of gB and gH was 30.8%, and the presence rates of either gB or gH in glioblastomas were 60.3%. While IDH R132H mutations were significantly correlated with a better clinical outcome (p=0.006), the presence of neither gB (p=0.551) nor gH (p=0.871) had prognostic values. Furthermore, there was no significant association between the presence of HCMV and gliomas' characteristics, neither with patients' age, gender, KPS, IDH mutations nor PTEN loss. In conclusion, our results support the fact that HCMV is detected in glioblastomas. However, no predictive value of HCMV was observed, the treatment of glioblastomas targeting HCMV was needed to be reevaluated by studies again.

Key words: glioblastoma, human cytomegalovirus, immunofluorescence, molecular pathology

Glioblastomas (GBM) are the most common malignant brain tumors with a median survival around 14–16 months [1]. Identification of glioma cancer stem cells (GCSC) is of great significance in underlining the aggressive behavior of glioblastomas [2]. Recent studies suggest a strong relationship between HCMV (Human Cytomegalovirus) infection and GCSC features of GBM [3, 4]. However, in 2002 was HCMV first detected in malignant gliomas, suggesting a role of HCMV in tumor genesis [5]. During the decades, many arguments arose out of presence of HCMV in blood and tumor tissue of glioblastomas, suspecting HCMV as a pathogenesis in glioblastomas [6–15]. The characteristics of HCMV infection has been well established that HCMV infection is closely correlated with regional economy, race and environment [5, 7, 8, 13, 14, 16, 17]. Additionally, HCMV infection is mostly prevalent in astrocytic gliomas and glioblastomas [18, 19]. The identification of IDH mutations in gliomas suggested that the genetic changes in two groups of gliomas were completely different [20]. IDH mutations were less common in glioblastoma than grade II–III gliomas. Also patients carrying IDH mutations had improved overall survivals [21, 22]. The classification of gliomas based on WHO 2007 pathology is presented with different clinical outcomes, when IDH mutations were involved [23, 24]. The updated WHO 2016 classification of glioblastomas included IDH mutations [25]. The gH and gB of the HCMV were two virion envelope proteins essential for the infection of host [26]. The two proteins were specific and used for the detection of HCMV [7, 27–29].

In this study, we detected the presence of HCMV with the immunofluorescence of gB and gH in glioblastomas. The relationship between HCMV presence and gliomas' characteristics was also analyzed here.

Patients and methods

Patients. There were 64 primary glioblastoma and 4 secondary glioblastoma patients involved in this study. All of them underwent a surgical treatment in Sanbo Brain Hospital from 2014 to 2015. Medical records like age, gender and Karnofsky Performance Status (KPS) scores were obtained in details. This study was approved by Ethics Committee of

Sanbo Brain Hospital. The written informed consents were obtained from all the patients involved in this study.

Immunohistochemistry (IHC). Immunostaining of IDH1 R132H mutations and PTEN were performed as in our previous research. Tumor samples were fixed with 10 % neutral buffered formalin, and further embedded in paraffin. Antigen retrieval was facilitated by heating, and endogenous peroxidases were neutralized with 3% hydrogen peroxide routinely [30]. Primary antibodies against IDH1 R132H (Dianova 1:100) and PTEN (ZSGB-BIO 1:150) were applied overnight at 4 °C. Poly-HRP Anti-Mouse/Rabbit IgG Detection System (PV-9000 ZSGB-BIO, China) was employed for 30 min at 37 °C. The cutoff values were 10% for IDH1 R132H, p53, MGMT and PDGFR, and 30% for Ki67.

Immunofluorescence (IF). Immunofluorescence was performed for the detection of CMV glycoprotein H (gH) and glycoprotein B (gB) in glioma tissues. All the experimental procedures were carried out in strict accordance with the manufacturer's instructions. Briefly, tumors were quickly frozen in liquid nitrogen and stored at –80 °C. Then, the tumor samples were cut into 7 μm thick sections, and the sections were thawed at room temperature prior to fixing in cold acetone for 10 minutes. A mouse monoclonal antibody against either CMV gH (sc-58113) or CMV gB (sc-70943) was purchased from SANTA CRUZ. Fluorescence microscope (BX-51 OLYMPUS JAPAN) was employed for the capture of the image.

Statistics. Student's t test, Chi Square and Kaplan–Meier Analysis were employed for data analysis, with the use of SPSS 22.0.

Table 1. Study population characteristics.

Variable	Number	%
Age		
mean	48.15±1.86	–
median	48	–
Gender		
female	33	48.53
male	35	51.47
KPS		
≥70	55	80.88
<70	13	19.12
Surgery		
non-GTR	21	30.88
GTR	47	69.12
Stupp protocol		
incomplete	14	20.59
complete	42	61.76
NA	12	17.65
IDH mutation		
mutant	13	19.12
wild-type	55	80.88
PTEN		
loss	11	16.18
presence	57	83.82

Results

Study characteristics. 68 patients were involved in this study, including 33 females and 35 males respectively. The follow-up was available in 59 cases. All the patients underwent an operation, and 42 of them postoperatively had a complete radiochemotherapy according to the Stupp's protocol [31] (Table 1).

Detection of HCMV. Immunofluorescence was used to detect the presence of HCMV in glioblastoma tissues. Representative images are shown in Figure 1. The gB and gH was detected in 33 (48.5%) patients and 29 (42.6%) patients, respectively. A strong correlation of gB and gH was also observed here (p=0.000).

Table 2. The relationship between HCMV infection and clinicopathological factors.

Factors		gB		p-value	gH		p-value	Diagnosis 1*		p-value	Diagnosis 2*		p-value
		+	–		+	–		+	–		+	–	
age	≤55	22	25	0.671	20	27	0.981	16	31	0.399	26	21	0.210
	> 55	11	10		9	12		5	16		15	6	
gender	female	16	17	0.994	13	20	0.598	10	23	0.920	19	14	0.656
	male	17	18		16	19		11	24		22	13	
KPS	≥ 70	26	29	0.670	24	31	0.734	18	37	0.731	32	23	0.464
	< 70	7	6		5	8		3	10		9	4	
IDH R132H	mutant	4	9	0.154	3	10	0.113	1	12	0.093	6	7	0.247
	wild-type	29	26		26	29		20	35		35	20	
PTEN	absence	5	6	0.824	3	8	0.428	2	9	0.523	6	5	0.929
	presence	28	29		26	31		19	38		35	22	

*The patients detected with both gB and gH were grouped as "Diagnosis 1". Patients who showed either gB or gH were grouped as "Diagnosis 2".

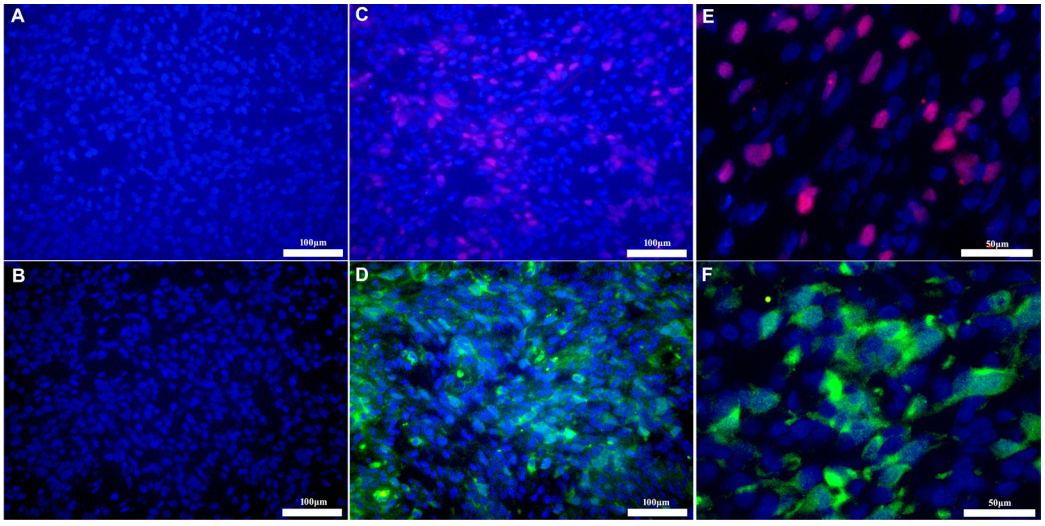

Figure 1. Immunofluorescence of gB and gH. Negative gB (A) and gH (B); positive gB (C) and gH (D); enlarged view of positive gB (E) and gH (F).

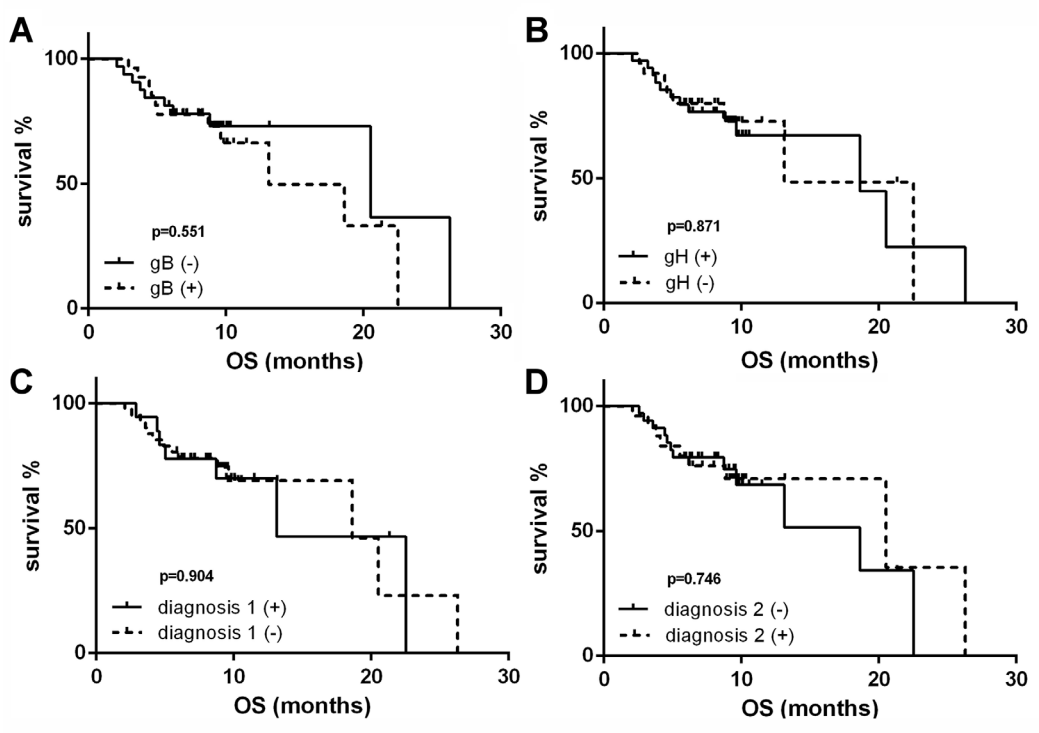

Figure 2. Kaplan–Meier survival curves according to the division of gB and gH. The clinical outcomes did not differ among the groups. The overall survival in GBM patients was not related to gB (A) or gH (B), Diagnosis 1 (C) or Diagnosis 2 (D).

To improve the specificity of detecting HCMV, we classified patients detected with both gB and gH as "Diagnosis 1". Patients who showed either gB or gH were grouped as "Diagnosis 2" for the sensitivity. However, we did not find any correlations between HCMV infection and patients' clinicopathological factors (Table 2).

Survival analysis. IDH-1^{R132H} mutations were significantly associated with longer OS [median 22.533 months (95% CI not available) vs. 13.133 months (95% CI 4.653–21.614); p=0.006, log rank test]. However, we did not find a predictive role neither of gB (p=0.551, log rank test, Figure 2A) nor of gH (p=0.871, log rank test, Figure 2B).

Then, we examined the predictive role of "Diagnosis 1" and "Diagnosis 2" in glioblastomas. No correlations of OS were found neither in "Diagnosis 1" (p=0.904, log rank test, Figure 2C) nor "Diagnosis 2" (p=0.746, log rank test, Figure 2D). We also detected that HCMV could predict the outcome in IDH-1 wt glioblastomas. There were no associations between the presence of HCMV and clinical outcome (data not shown).

Discussion

Herein, our study proved the infection of HCMV in glioblastoma tissues with the method of IF. However, there were many arguments addressing HCMV presence in tumor tissue and blood detected by various methods including PCR/FISH/IHC [18]. In Lau Et Al's study, no HCMV gB was found in GBM, AA, A or OA.[29] The same results were also observed in 77 brain tumors, including II–IV gliomas [28]. A study from Japan also found that HCMV gB was not present in 40 glioblastomas [9]. On the contrary, the positive rates of HCMV gB were reported to be 40.9% for grade II, 53.8% for grade III, and 61.7%~63.1% for grade IV [7, 27]. Our research showed that 48.5% (33/68) and 42.6% (29/68) of the patients were found positive for HCMV gB or gH, respectively. The co-occurrence of gB and gH was found in 21 patients (30.8%). The presence of either gB or gH was detected in 41 patients (60.3%). However, no relationship was found between HCMV presence and glioma's characteristics, neither with patients' age, gender, KPS, IDH-1^{R132H} mutations nor PTEN loss. Consequently, our results supported the fact that HCMV was present in glioblastomas. The mechanism was likely to be angiogenesis, immunosuppression, promotion of cell proliferation, tumor invasion, and inhibition of apoptosis and autophagy [32].

Additionally, the presence of HCMV was not likely to be correlated with clinical outcome in our study, which was consistent with the previous data [27]. In Ding Et Al's study, IHC was used to detect HCMV proteins IE1-72 and pp65, and PCR was adopted to measure HCMV DNA both in tumor and blood. However, none of these glioblastoma components were associated with clinical outcomes [27]. However, low GBM-infection was associated with better clinical outcomes according to several reports [33, 34]. The reasons for the inconsistent results are not clear now. However, Valganciclovir as an added-on immunotherapy within 6-months post operationally showed beneficial effect in killing tumor cells in glioblastomas. However, prolonged overall survival (OS) was not found due to the use of Valganciclovir [35, 36]. On the contrary, the continuous usage of Valganciclovir exceeding 6 months seemed to improve OS in glioblastomas [36]. Consequently, targeting HCMV in treating glioblastomas was needed to be further investigated.

In conclusion, our results favor that HCMV is present in glioblastomas. However, no relationship between HCMV presence and patients' prognosis was found.

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