Original article

Antiviral resistance in human cytomegalovirus due to *UL54* mutations without *UL97* mutations

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Abstract

Background: The concurrent detection of human cytomegalovirus (CMV) with *UL97* and *UL54* mutations is crucial for prescribing adequate antiviral treatment when drug-resistant CMV infection is suspected. We investigated the frequency of resistance-conferring mutations among patients with persistent or recurrent CMV infection and further reviewed the subgroup with *UL54* mutations without *UL97* mutations.

Methods: Patients with persistent or recurrent CMV infection after 4 weeks of treatment with ganciclovir or foscarnet were consecutively enrolled between November 2012 and May 2019. The direct sequencing of *UL97* and *UL54* was performed to detect resistance mutations in CMV.

Results: A total of 101 sequencing datasets were obtained from 65 enrolled patients. CMV *UL97* and *UL54* mutations were detected in 15.4% (10/65) and 9% (6/65) of patients, respectively. The CMV retrieved from two patients (3%) had mutations in both genes. Four patients with CMV *UL54* mutations alone had a history of haploidentical peripheral blood stem cell transplantation, and foscarnet was administered for over 4 weeks to these patients; 21.5% of patients had suspected resistant CMV infection with either *UL97* or *UL54* mutations.

Conclusion: In this study, CMV *UL54* mutations but not *UL97* mutations were found in patients subjected to prolonged foscarnet administration for CMV disease.

Keywords: Antiviral resistance, Cytomegalovirus, *UL54*, *UL97*

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Introduction

Human cytomegalovirus (CMV) disease is commonly treated with ganciclovir (GCV) or its oral prodrug, valganciclovir. Second-line treatment drugs for CMV disease, including foscarnet (FOS) and cidofovir (CDV), target UL54 DNA polymerase. Prolonged antiviral therapy can lead to mutations in the *UL97* and/or *UL54* genes [1]. CMV resistance to these drugs is uncommon, ranging from 0.4% to 11.9% in solid organ transplant patients and 1% to 5% in patients who have undergone hematopoietic stem cell transplant [2,3]. However, drug-resistant CMV infection is associated with inferior prognosis in these two patient groups [2,4].

UL97 mutations are well-known as the most prevalent causes for CMV resistance [5,6]. A previous study [6] showed that approximately 30% of 570 samples submitted to a commercial laboratory in the US were positive for *UL97* or *UL54* gene mutations, and 62.5% of 64 samples with *UL54* mutations also possessed

UL97 mutations. Therefore, *UL97* mutations are primarily tested in patients suspected to be infected with resistant strains of CMV, and *UL54* mutations are tested concurrently or only in *UL97* mutation-negative cases. *UL54* mutations, which usually appear after *UL97* mutations, are associated with resistance to not only the first-line drug GCV, but also to the other second-line agents such as CDV and FOS [7]. Therefore, *UL54* as well as *UL97* mutations should be investigated in CMV patients suspected with antiviral resistance. Here, we describe the frequency of CMV resistance-related gene mutations among patients with persistent or recurrent CMV infection, and further reviewed the subgroup possessing *UL54* mutations without *UL97* mutations.

Materials and methods

Patients suspected to be infected with resistant CMV strains following persistent or recurrent CMV detection after 4 weeks of antiviral therapy using GCV or FOS were subjected to *UL97* and *UL54* mutation testing in a consultation-based setting between November 2012 and May 2019 at the Asan Medical Center. Clinical and laboratory data were obtained from the hospital's electric medical records and laboratory information system. CMV polymerase chain reaction (PCR) was performed with Artus CMV PCR Test (QIAGEN Gaithersburg, Inc., Gaithersburg, MD, USA) using a Rotor-Gene Q (Qiagen Inc., Hilden, Germany), and the threshold of CMV detection was set as 2.42 log copies/mL. In September 2018, Artus CMV PCR Test was replaced with Abbott RealTime CMV Assay (Abbott Molecular Inc., Des Plaines, IL, USA) with the limit of detection value set at 1.49 log IU/mL. Direct sequencing of *UL97* and *UL54* genes was performed for detecting the CMV resistance mutations as described previously [8,9]. Variants were detected by comparing with the reference strain AD169 (GenBank accession number BK000394). Detected variants were analyzed along with the previously reported ones and synonymous variants were not analyzed [10,11]. Categorical data were analyzed with chi-square test using MedCalc (version 20.011; MedCalc Software, Ostend, Belgium).

Results

A total of 101 CMV DNA samples from 65 patients were subjected to CMV resistance testing. The median age of study patients was 19 years and 62% of them were male. 75% of study patients had underlying disease of hematologic malignancy. All mutations in *UL97* or *UL54* genes were identified in 14 patients (21.5%). 10 (15.4%) and 6 (9.2%) patients exhibited mutations in the *UL97* and *UL54* genes, respectively. Observed mutations from both genes are presented in Table 1. Between patients with and without any mutation in *UL97* and *UL54* genes, there was no significant difference of underlying disease of hematologic malignancy (P=0.31) and sex (P=0.32). Changes in the test numbers of samples and % sample harboring any mutation were not significant during the study period; the test numbers of samples (% sample harboring any mutation) were 5 (0%) in 2012, 4 (25.0%) in 2013, 12 (8.3%) in 2014, 18 (16.7%) in 2015, 15 (13.3%) in 2016, 17 (23.5%) in 2017, 26 (26.9%) in 2018, and 4 (25.0%) in 2019.

Table 1. Human cytomegalovirus *UL97/UL54* gene mutations observed in this study

Case No.	Year	*UL97* mutations (specimen)	*UL54* mutations (specimen)	Viral loads
3	2019	A594P (blood)	-	4.12 log IU/mL
6	2018	-	F412L (blood)	3.03 log IU/mL
7	2018	L595F (blood)	N408D (blood)	4.40 log copies/mL
11	2017	-	V715M (blood)	3.34 log copies/mL (blood)
14	2018	-	L501I (CSF)	4.73 log copies/mL (CSF)
19	2017	-	L802M (CSF)	5.92 log copies/mL
22	2017	505-506 in frame deletion (anterior chamber fluid)	V787L (blood)	4.72 log copies/mL
29	2017	C603W (blood)	-	N/A
32	2016	C603W (blood)	N408D/P522S (blood)	3.57 log copies/mL
40	2016	M460V (blood)	-	5.69 log copies/mL
46	2015	A594V (blood)	-	4.12 log copies/mL
55	2015	597-600 in frame deletion (blood)	-	2.42 log copies/mL
60	2014	L595S (blood)	-	2.42 log copies/mL
65	2013	M460I (blood)	-	4.59 log copies/mL
40	2019	M460V (blood)	-	3.99 log IU/mL
46	2015	A594V (blood)	-	2.42 log copies/mL
55	2014	597-600 in frame deletion (blood)	-	2.42 log copies/mL
60	2013	L595S (blood)	-	4.59 log copies/mL
65	2019	M460I (blood)	-	3.99 log IU/mL

Abbreviations: CSF, cerebrospinal fluid; N/A, not available.

Four cases had *UL54* mutations alone. Patient characteristics and CMV kinetics of these cases are described in Table 2 and Fig. 1, respectively. They all had a history of haploidentical peripheral blood stem cell transplantation (HaploPBSCT) and FOS was administered to these four patients before the detection of variants.

Table 2. Patient characteristics of four cases with *UL54* mutations alone

Patient No.	Sex	Age (yr)	Underlying disease	CMV disease	Outcome	*UL54* mutations
6	Female	15	MDS, HaploPBSCT	Pneumonia Retinitis Encephalitis	Expired	F412L
11	Female	18	AA, HaploPBSCT	Pneumonia Encephalitis	Expired	V715M/L501I
14	Female	18	AA, HaploPBSCT	Encephalitis	Improved	L802M
19	Female	19	AA, HaploPBSCT	Retinitis	Improved	V787L

Abbreviations: CMV, cytomegalovirus; MDS, myelodysplastic syndrome; HaploPBSCT, haploidentical peripheral blood stem cell transplantation; AA, aplastic anemia.

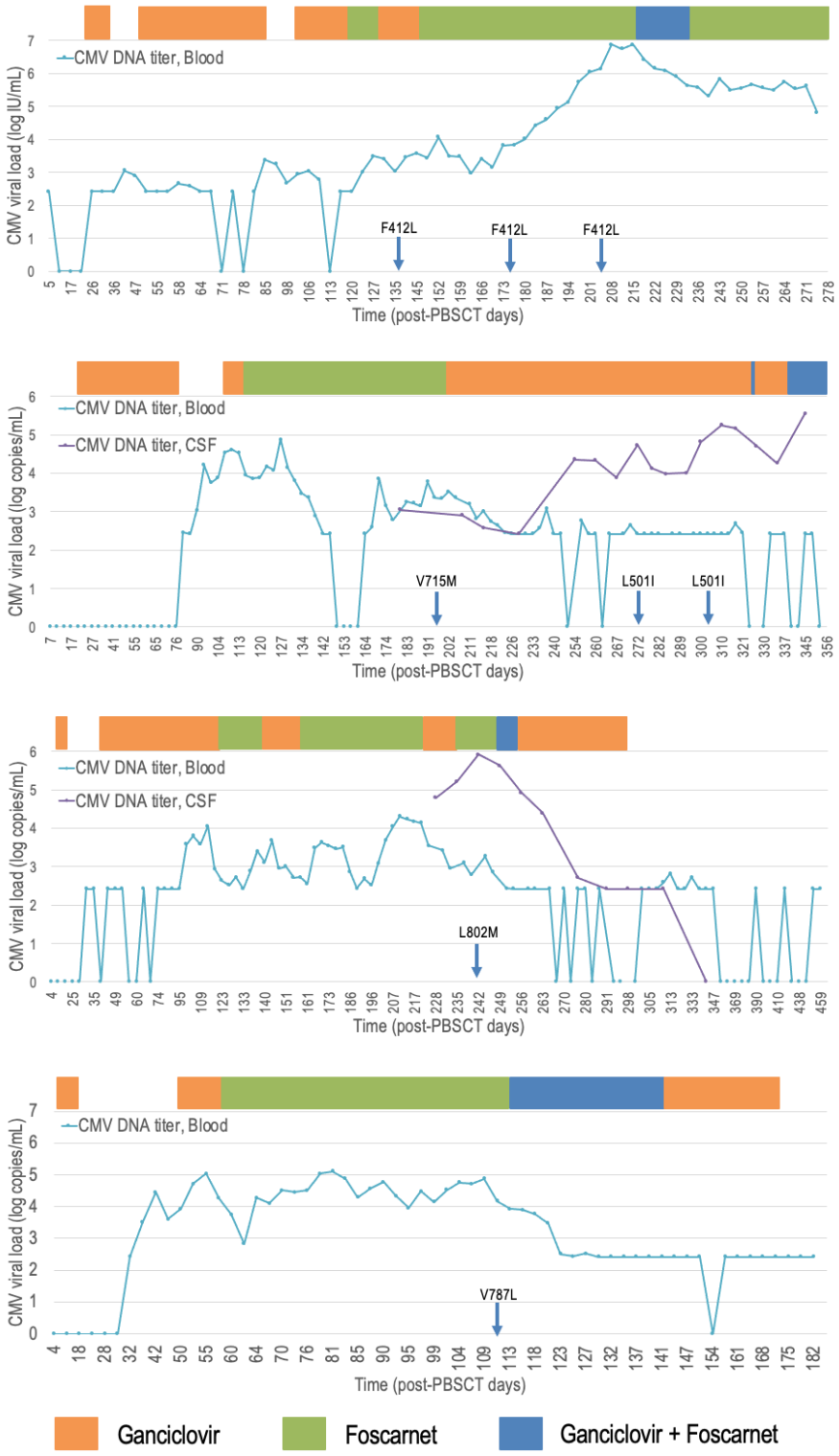

Fig 1. CMV kinetics of four cases with *UL54* mutations alone (Patients #6, #11, #14, #19). Bar graph indicates the duration of antiviral drug administration. Blue arrows indicate the specimen dates of CMV viruses with specified *UL54* mutations. CMV, cytomegalovirus; PBSCT, peripheral blood stem cell transplantation; CSF, cerebrospinal fluid.

Discussion

Approximately, one-fifth of the patients with persistent or recurrent CMV exhibited at least one mutation in *UL97* and *UL54*. High prevalence of CMV mutations was mainly due to the inclusion criteria of patients with persistent or recurrent CMV after 4 weeks of antiviral treatment using GCV or FOS. Resistance emergence varied along with organ type among solid organ transplantation (SOT) patients [3]; thus, the composition of the included SOT patients could affect the mutation prevalence in this study.

All except two mutations were well-known mutations [12-14]. The two unknown mutations are both in-frame deletions. In-frame deletion of codon 597-600 is within the hot-spots for GCV resistance (codons 460, 520, and 590-607) [12]. In-frame deletion of the *UL97* gene, especially at codons 591 to 603 is less-common but has been reported continuously, and some reported these mutations to confer 4- to 10-fold resistance [13]. The other one was an in-frame deletion of codons 505-506 located on the kinase domain, which is not a well-known mutation or polymorphism [14], but a variant of unknown significance.

All cases with *UL54* mutations alone were related to HaploPBSCT and exhibited a prolonged treatment history with FOS. A previous study reported three patients with *UL54* mutations alone, who were hematopoietic stem cell transplantation recipients, and all were treated with both GCV and FOS [15]. In line with this, prolonged use of FOS is possibly related to CMV resistance conferred by *UL54* mutations without *UL97* mutations. This finding highlights the importance of testing *UL54 mutations* especially in patients treated with FOS.

Our study has several limitations due to its observational nature. Furthermore, our study population was limited to patients who were clinically suspected to have resistant CMV infection, and therefore, some patients subjected to more than 4 weeks of antiviral therapy for CMV treatment were probably excluded. Additionally, the number of patients infected with drug-resistant CMV in one institution was relatively small.

Conclusively, 21.5% of patients suspected with resistant CMV infection possessed one of the two *UL97* and *UL54* mutations. In addition, *UL54* mutations without *UL97* mutations was found in patients subjected to prolonged administration of FOS for CMV disease.

Ethics statement

The Institutional Review Board (IRB) of the Asan Medical Center (No. 2012-0401) approved this study.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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