



Short Communication

Genotypic characterization of herpes simplex virus DNA polymerase UL42 processivity factor

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ABSTRACT

The herpes simplex virus (HSV) DNA polymerase is composed of the UL30 catalytic subunit and the UL42 processivity factor. The UL42 subunit increases the processivity of the polymerase along the DNA template during replication. The molecular mechanisms of HSV resistance to drugs interfering with viral DNA synthesis reported so far mainly rely on modifications of the viral thymidine kinase and DNA polymerase. We aimed to extensively describe the genetic variations of HSV UL42 processivity factor and to evaluate its potential involvement in resistance to antivirals. The full-length UL42 gene sequence of HSV was investigated among two laboratory strains (KOS and gHSV-2), 94 drug-sensitive clinical isolates and 25 phenotypically ACV-resistant clinical isolates. This work provided extensive data about natural variability of UL42 processivity factor among both HSV-1 and HSV-2 strains and showed that this viral protein is highly conserved among HSV strains, with a weaker variability for HSV-2. The analysis of 25 HSV clinical isolates exhibiting ACV-resistance documented most of the previously reported mutations related to UL42 natural polymorphism in addition to some unpreviously described polymorphisms. Surprisingly, a single-base deletion in UL42 gene sequence leading to a frameshift in the C-terminal region was identified among 3 HSV clinical isolates. From this preliminary study, UL42 processivity factor did not seem to be likely involved in HSV resistance to antivirals.

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Herpes simplex virus (HSV)-1 and HSV-2 might be associated with severe morbidity and mortality in immunocompromised individuals, in particular transplant recipients and AIDS patients (Dignani et al., 2002; Englund et al., 1990; Hill et al., 1991). The drugs currently used for the treatment of HSV infections, acyclovir (ACV) and its prodrug valacyclovir, foscarnet (FOS) and cidofovir (CDV), have provided a major advance in the management of HSV diseases (Gilbert et al., 2002). However, the antiviral effectiveness may be restricted due to significant toxicity or the emergence of viral drug-resistance. HSV resistance to ACV and FOS has been associated with modifications located in the UL23 thymidine kinase (TK) and UL30 DNA polymerase. ACV-resistance mainly stems from TK alterations due to the occurrence of either a shift of the translational reading frame or non-synonymous mutations. Resistance to FOS, and to a lesser extent to ACV, is related to amino acid changes within catalytic or conserved regions of the DNA polymerase (Burrel et al., 2010; Gilbert et al., 2002). As for other herpesviruses, HSV DNA polymerase complex consists of a hetero-

dimer composed of a catalytic subunit (UL30) and an accessory protein (UL42) which increases the processivity of the enzyme (Loregian et al., 2000). UL42 processivity factor, consisting of a 488 and 470-amino-acid protein for HSV-1 and HSV-2, respectively, tethers DNA tightly as a monomer allowing prolonged association, thereby conferring extended DNA strand synthesis. Crystal structure data evidenced that the UL30/UL42 protein–protein interaction involves several specific residues located within the UL30 extreme C-terminal domain (codons 1200–1235) and the UL42 connector loop (codons 160–175) (Zuccola et al., 2000). The disruption of this interaction has been proposed as a potential target to hinder viral replication using oligopeptides corresponding to the very C-terminal region of the UL30 or peptidomimetic compounds (Loregian and Palu, 2005). Hitherto, only the identification of the UL30 and UL42 regions responsible for the physical and functional interaction of these two proteins has been the subject of extensive investigations. The aims of this work were to describe precisely the entire natural polymorphism of HSV UL42 processivity factor and to perform its genotypic characterization among drug-sensitive and drug-resistant HSV clinical isolates in order to evaluate its potential involvement in resistance to antivirals.

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Two laboratory strains (KOS and gHSV-2), 94 drug-sensitive (43 HSV-1 and 51 HSV-2) and 25 phenotypically drug-resistant clinical isolates were studied. The genotypic data concerning UL23 and UL30 genes have been previously described (Burrel et al., 2010). Viral DNA was extracted from viral stocks using QIAamp® DNA blood mini kit (Qiagen, Courtaboeuf, France). The full-length UL42 gene from HSV-1 (1467 base pairs [bp]) and HSV-2 (1413 bp) was amplified by nested PCR using the proofreading enzyme Expand High Fidelity (Roche, Meylan, France). Amplified products were sequenced using overlapping primer pairs with the Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Courtaboeuf, France) and analyzed with the automated sequencer ABI 3730 Genetic Analyzer (Applied Biosystems). Primers used for gene amplification and sequencing are listed in Table 1. All nucleotide and amino acid sequencing results were compared with published sequences of the reference strains 17 (HSV-1) and HG52 (HSV-2) (GenBank accession numbers X14112 and Z86099, respectively) using Seqscape v2.5 software (McGeoch et al., 1985, 1987). All UL42 sequences determined in this study have been deposited in the GenBank database under accession numbers JF810705 through JF810823.

Among HSV drug-sensitive strains (2 laboratory strains and 94 clinical isolates), the interstrain identity at the nucleotide level of UL42 gene ranged from 98.8% to 99.8% and from 99.3% to 99.9% for HSV-1 and HSV-2, respectively. In comparison with reference sequences, 58 and 22 nucleotide substitutions were identified within the coding sequence for HSV-1 and HSV-2, respectively, corresponding to an average number of 9.1 and 4.4 per strain. Almost half of them (35/80) were synonymous polymorphisms. At the amino acid level, the interstrain identity of UL42 processivity factor was >98.4%, with the identification of 28 and 12 amino acid changes for HSV-1 and HSV-2, respectively, corresponding to 5.5% and 2.6% of the total codons of the protein. The number of amino acid changes per strain ranged from 1 to 7 and from 0 to 4 for HSV-1 and HSV-2, respectively. As presented in Table 2, the most frequent amino acid changes observed were P13H, G284A, L377S, H378Y and R404H for HSV-1, S328T, S351F and P389A for HSV-2. Surprisingly, one HSV-1 isolate harbored a guanosine deletion (Del 1G 1433) leading to the creation of a frameshift and the alteration of the extreme C-terminal amino acid sequence from the residue 479. All these changes were located outside conserved domains of the viral protein, except R404H and K411R (HSV-1), P389A and A399T (HSV-2), which were located within the conserved nuclear localization signal (NLS) of UL42 processivity factor (Fig. 1). Among the 25 drug-resistant HSV strains, the amino acid

sequences were identical to those of reference strains for 3 isolates. For the other isolates, 15 out of the 40 amino acid changes previously evidenced in HSV drug-sensitive isolates were found. Moreover, new amino acid changes were identified: S21P, K105T, A353S, A419T, S434P and R397C for HSV-1, G93E and F457S for HSV-2 (Table 2). These changes were located outside the conserved/functional domains of the protein, except K105T and R397C (HSV-1) settled in the back face interacting with DNA and in the NLS, respectively (Figs. 1 and 2). One isolate harbored the association of 3 amino acid changes unpreviously described (A353S + A419T + S434P) (Table 2). For one HSV-1 resistant isolate, the frameshift deletion previously described Del 1G 1433 was found. One HSV-2 isolate also harbored a frameshift deletion (Del 1C 1185) affecting NLS motif from residue 389 (Fig. 1). Regarding HSV-1, one isolate exhibited the unpreviously described R1229I change located in C-terminal domain of UL30 DNA polymerase (data not shown) (Burrel et al., 2010). The intermolecular hydrogen bond involving this residue and Q171 residue in UL42 processivity factor is fundamental for the UL30/UL42 interaction (Fig. 2) (Loregian and Palu, 2005; Zuccola et al., 2000). No specific association of mutation within UL42 processivity factor and drug-resistance mutations within TK and/or DNA polymerase could be evidenced (data not shown). However, the use of a proofreading high fidelity enzyme for UL42 gene amplification cannot definitively rule out the possibility that some of the mutations described above might be the consequence of PCR artefacts.

This study aimed to analyze the natural variation of HSV UL42 processivity factor and its potential involvement in HSV resistance to antivirals. This work provides extensive data about UL42 natural polymorphism of both HSV-1 and HSV-2. Our results evidenced that UL42 processivity factor is highly conserved among 96 HSV drug-sensitive strains (2 laboratory strains and 94 clinical isolates), with an interstrain identity >98.4% at both nucleotide and amino acid levels, and a weaker variability for HSV-2. Similar results were previously reported for the UL30 catalytic subunit of HSV DNA polymerase (Burrel et al., 2010; Chiba et al., 1998). Given their essential role in replication, it appears logical that both UL42 and UL30 proteins are extremely conserved. Moreover, it has been reported that DNA polymerase processivity factors from human cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) (encoded by UL44 and U27 genes, respectively) also exhibited a low natural polymorphism (Boutolleau et al., 2009; Bonnafous et al., 2010). Regarding the 25 drug-resistant HSV isolates, 15 natural polymorphisms previously identified in the first part of our study were found (Table 2). However, 9 unpreviously described changes

Table 1
Primers used for amplification and sequencing of UL42 gene among HSV-1 and HSV-2 strains.

HSV type	Function	Name	Sequence (5' → 3')	
HSV-1	First-round PCR (outer primers)	42H1-F1	R: CCTGGCTGCCATCAAAAC	
		42H1-R1	F: GGCACACACATGAACACAC	
	Second-round PCR (inner primers)	42H1-F2	F: AAGCCACCTCGTCACTGT	
		42H1-R2	R: GACACGCGGGAAGTGTT	
	Sequence reaction	42H1-A	R: CGTTAAGGACCTTGGTGAGC	
		42H1-B	F: CAGCGAGACGCTGATGAAG	
		42H1-C	R: GGAGTCTCGGCTGTCTGTTG	
		42H1-D	F: TGAACCCAGAAAGATTGG	
		+42H1-F2 and 42H1-R2		
HSV-2	First-round PCR (outer primers)	42H2-F1	F: CTCGGGTAGCCACTGCTCT	
		42H2-R1	R: AACACACACGACGCCATA	
	Second-round PCR (inner primers)	42H2-F2	F: CTCGTCTCTGCGGATTGTC	
		42H2-R2	R: GGGGACACACTACCCCATTT	
	Sequence reaction	42H2-A	R: CGTTCACCCTTCGTGAG	
		42H2-B	F: CGAGCTTCGCGGTACTACTC	
		42H2-C	R: AGGAGGAGTCTGGCTGTCT	
		42H2-D	F: AGCGGCTGCGCTGAACT	
		+42H2-F2 and 42H2-R2		

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