Human herpesvirus 6A U27 plays an essential role for the virus propagation

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学 位 論 文 の 内 容 要 旨

Human herpesvirus 6A U27 plays an essential role for the virus propagation

ヒトヘルペスウイルス 6A U27 遺伝子はウイルス感染に不可欠である

神戸大学大学院医学研究科医科学専攻

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ANNA LYSTIA POETRANTO

アンナ リスティア ポートラント

(Introduction)

Human herpesvirus 6 (HHV-6) belongs to the Roseolavirus genus of betaherpesvirus subfamily. Based on the distinct genetic, biological and epidemiologic differences, HHV-6 was classified into two species, HHV-6A and HHV-6B. The HHV-6 gene U38 encodes the DNA polymerase which synthesizes the nascent DNA, while HHV-6 U27 gene encodes the processivity factor which tethers the U38 on the template DNA not to dissociate during the enzyme action. The HHV-6 U27 encodes 41-kDa nuclear phosphoprotein and binds to DNA and the U38. The interaction between U27 with DNA polymerase has been reported to increase the DNA synthesis activity by in vitro DNA synthesis assay.

The previous reports of herpesvirus processivity factors such as UL42 in HSV-1, BMRF1 in Epstein-Barr virus (EBV), UL44 in HCMV, and U27 in HHV-7, have been shown to be important for viral replication. The processivity factors are required for herpesvirus viral replication to overcome the tendency of DNA polymerase to dissociate from the template. Meanwhile, the role of U27 during the viral replication process of HHV-6 has not been reported yet.

(Results/Discussion)

1. Design of the frameshift mutation at the HHV-6A U27 gene

To design an HHV-6A genome DNA with a U27-deficient mutation, we referred to the amino acid sequence of HCMV UL44, which has high homology (43%) with HHV-6A U27. Because the crystal structures of HCMV UL44 revealed that the core protein fold of UL44 starts at residue E9 and corresponds to E23 of U27, we decided to insert an additional nucleotide within the corresponding region of U27, which was expected to be involved in the protein fold. The insertion was located after K33, and caused a frameshift with stop codons.

2. Construction of HHV-6ABACU27mut and HHV-6ABACU27mutRev

The mutant and revertant were constructed using the HHV-6A BAC system, and two-step red-mediated recombination processes in *Escherichia coli* GS1783. The target site and the flanking regions are labeled regions 1, 2, 3, and 4, with an insertion point in region 3. The kanamycin-resistant gene fragment a*phAI*, which is flanked by U27 sequences, was amplified, then inserted at the target site by homologous recombination. The *aphAI* fragment was then excised by cleavage using *I-Sce*1, leaving the inserted nucleotide in the U27 gene. We named the resulting BAC DNAs HHV-6ABACU27mut and HHV-6ABACU27mutRev.

To eliminate the possibility of large modifications in the HHV-6A genome structure during recombination, we performed restriction enzyme digestion analysis using *Eco*RI and *Hind*III on the purified BAC DNAs of the mutant and revertant. According to *Eco*RI

digestion analysis, the insertion of the a*phAI* fragment in the wild-type HHV-6ABAC or HHV-6ABACU27mut resulted in the emergence of a 4,818-bp fragment and the loss of a 3,781-bp fragment. The corresponding changes are apparent in the results of the analysis, although they are not very clear owing to the overlap with other fragments. However, *Hind*III digestion clearly resulted in the expected removal of the 18,703-bp fragment and the addition of a 16,704-bp fragment due to the insertion of the *aphAI* fragment. No other differences were observed during a*phAI* insertion or removal, and the digestion patterns of the BAC DNAs were the same for HHV-6ABAC, HHV-6ABACU27mut, and HHV-6ABACU27mutRev. The mutation and the restoration of the wild-type sequence at the mutation position were confirmed by sequence analyses of HHV-6ABACU27mut and HHV-6ABACU27mutRev.

3. U27 deficient HHV-6A could not be reconstituted from HHV-6ABACU27mut

We can see the GFP expression JJhan cells transfected with BAC DNAs of HHV-6ABAC (wild type), HHV-6ABACU27mut, or HHV-6ABACU27mutRev at 48 hr post-transfection, indicating the cells have acquired the BAC DNAs. We then co-cultured the JJhan cells with CBMCs to propagate the reconstituted virus. We could observe that the GFP-positive cells transfected with the HHV-6ABAC and HHV-6ABACU27mutRev dramatically increased. In contrast, GFP signal in the cells transfected with the HHV-6ABACU27mut gradually decreased and eventually disappeared.

We confirmed whether the anti-U27 antibody can recognize the HHV-6A U27 expressed in the 293T cells with the HHV-6A U27 expression vector. The detected position was consistent with the size of U27, 41 kDa, as reported previously. Next, we detected the U27 and other viral proteins in the cells infected with viruses reconstituted from the HHV-6ABAC or HHV-6ABACU27mutRev DNAs. As shown in Figure 5B, we could detect the viral proteins U11, gQ1 and U27 in the cell lysate for HHV-6ABACU27mutRev as well as in that of HHV-6ABAC.

(Conclusion)

In the present study, we attempted to generate a U27-deficient virus based on the HHV-6ABAC system by introducing a frameshift mutation within the expected core protein fold of U27. The mutant BAC DNA—i.e., HHV-6ABACU27mut—was constructed as designed. However, the HHV-6A virus could not be reconstituted from the mutant BAC DNA, but could be reconstituted from the original HHV-6ABAC and from the revertant HHV-6ABACU27mutRev. This indicates that U27 is essential for the reconstitution of the virion and the viral life cycle of HHV-6A. For the first time, we demonstrated the critical role of U27 in the context of the HHV-6A viral genome. The analysis of mutant viruses has revealed that the processivity factors for HCMV, EBV, and HSV-1 are essential, as also demonstrated for HHV-6A in the present study. This suggests that processivity factors are one of the fundamental requirements of herpesviruses. The significance of HHV-6B U27 is still unclear because no BAC system is available; we suggest that U27 is also important for the viral life cycle of HHV-6B, considering the high homology (95%) between HHV-6A U27 and HHV-6B U27.

The function of HHV-6A U27 as a processivity factor has been investigated; it works in concert with the viral DNA polymerase U38. Although U38 is responsible for catalytic activity, U27 is also important for viral replication, as demonstrated here. It is worth noting that the U27 homolog in HCMV—i.e., UL44—has the additional function of downregulating the innate immune response by interfering with the IRF3 and NF-κB signaling pathway. Therefore, it is also possible that such extra functions of U27, if any, may also be important.

The processivity factors of herpesviruses—including HHV-6A U27—are candidate drug targets. When the interaction between the processivity factor and the DNA polymerase is impeded, the virus loses its ability to replicate. Therefore, detailed analysis of HHV-6A U27 will provide fundamental information for the development of an antiviral. The U27-deficient BAC DNA constructed in the present study—i.e., HHV-6ABACU27mut—will open the way to further investigations, such as complementation experiments using expression vectors of U27 mutants, or processivity factors of other herpesviruses.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第3002号	氏 名	ANNA LYSTIA POETRANTO
論文題目 Title of Dissertation	Human herpesvirus 6A U27 plays an essential role for the virus propagation ヒトヘルペスウイルス6A U27遺伝子はウイルス感染に不可欠である		
審査委員 Examiner	主 査 Chief Examiner 勝二郁夫 副 査 Vice-examiner 中村俊一 副 査 Vice-examiner 古屋敷智之		

（要旨は１，０００字～２，０００字程度）

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The processivity factors of herpesviruses—including HHV-6A U27—are candidate of drug targets. When the interaction between the processivity factor and the DNA polymerase is impeded, the virus loses its ability to replicate. Therefore, detailed analysis of HHV-6A U27 will provide fundamental information for the development of an antiviral. The U27-deficient BAC DNA constructed in the present study—i.e., HHV-6ABACU27mut—will open the way to further investigations, such as complementation experiments using expression vectors of U27 mutants, or processivity factors of other herpesviruses.

The candidate, having completed studies on HHV-6A, with a specialty in the role of U27 for viral replication, and having advanced the field of knowledge in the area of Medical Virology, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).