Human herpesvirus 6A nuclear matrix protein U37 interacts with heat shock transcription factor 1 and activates the heat shock response

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学 位 論 文 の 内 容 要 旨

Human herpesvirus 6A nuclear matrix protein U37 interacts with heat shock transcription factor 1 and activates the heat shock response

ヒトヘルペスウイルス 6A 核タンパク質 U37 は、heat shock factor 1 と相互作用し、heat shock 応答を活性化する

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1. Introduction

Human herpesvirus 6A (HHV-6A) is a dsDNA virus belonging to the *Roseolovirus* genus within the *Betaherpesvirinae* subfamily. It is frequently found in patients with neuroinflammatory disease, although its pathogenetic role, if any, awaits elucidation.

Nuclear egress complex (NEC), which consists of the nuclear matrix protein and the nuclear membrane protein, which is required for nascent nuclear capsid export by budding through the inner nuclear membrane into the perinuclear space between the inner and outer nuclear membranes. Here, we focus on the role of the HHV-6A nuclear matrix protein U37 in intracellular signaling.

The heat shock response is important for cell survival under stressful conditions that disrupt homeostasis, which is regulated by heat shock transcription factor 1 (HSF1). The heat shock response induces expression of heat shock proteins (HSPs), such as Hsp27, Hsp40, Hsp70 and Hsp90, most of which are molecular chaperones that function to prevent protein misfolding and aggregation. In infected cells, HSPs are known to be important for the precise function of viral proteins. In this present study, we focus on a novel function of nuclear matrix protein U37 in heat shock signaling.

Materials and Methods

To determine the role of U37 in heat shock signaling, initially, we conducted a luciferase reporter assay in HEK293T cells by transfecting the cells with plasmids carrying U37 together with a set of firefly luciferase reporter plasmids harboring responsive element for HSE, NF-κB, IFN-β, and CRE. To confirm the specificity of U37 in such signaling, another round of luciferase reporter assay was performed with inhibitor. We also included homologue protein of human cytomegalovirus, belonging to the *Betaherpesvirinae* subfamily. To determine whether U37 induced HSPs accumulation, we performed immunofluorescent assays. To investigate whether U37 activates HSF1, we performed immunoblotting using antibody against phosphorylated HSF1. The interaction between U37 and HSF1 is confirmed by NanoBit assay as well as immunofluorescence assay. Finally, we focused on the importance of heat shock signaling in HHV-6A replication by analyzing the virus protein accumulation and genome copy number in virus infected cells under drug treatment including HSPs and HSF1 inhibitors.

2. Results

HHV-6A U37 activates the HSE promoter.

Ectopic expression of HHV-6A U37 significantly stimulated the activity of HSE-luc. Of note, heat shock increased the HSE-luc activity in the presence or absence of HHV-6A U37, suggesting that

heat shock and HHV-6A U37 synergistically activate the HSE promoter. Furthermore, accumulation of Hsp90 was significantly increased in Flag-HHV-6A U37-expressing cells compared to the mock transfected cells. These results suggest that HHV-6A U37 specifically activates the cellular heat shock response system.

HSE is not activated by HHV-6A U34/U37 complexes or by HCMV UL53.

To investigate the region in HHV-6A U37 responsible for HSE stimulation, we constructed a series of truncated HHV-6A U37s and analyzed their effects on HSE-luc. As result, C-terminal region of HHV-6A U37 was deleted, still enhanced HSE-luc to similar levels as wild-type U37. In contrast, HHV-6A U37 90-264, in which the N-terminal region of HHV-6A U37 was deleted, was expressed properly but failed to activate the HSE, similar result is obtained when U34/U37 complexes is expressed. These results indicate that the N-terminal region of HHV-6A U37 is important for activation of the HSE. By the other hand, ectopic expression of HCMV UL53, which is the homologue of HHV-6A UL37, did not increase HSE-luc activity in the presence or absence of the other NEC component HCMV UL53. These results indicate that the ability of HHV-6A U37 to stimulate the HSE is not conserved in the other herpesviruses.

The role of HSF1 in activation of the HSE promoter by HHV-6A U37.

HEK293T cells were transfected with the expression plasmid for HSF1 in the presence or absence of plasmids expressing HHV-6A U37. The phosphorylation of HSF1 at Ser-326 was enhanced in cells transfected with the HHV-6A U37 expression plasmid compared to control, whereas the accumulation of HSF1 was not affected. This finding suggests that HHV-6A U37 mediates activation of HSF1. Next, interactions between HHV-6A U37 and HSF1 were investigated by the NanoBiT assay, which enables measurement of protein-protein interactions in cells. Co-expression of HHV-6A U37 and HSF1 significantly increased luminescence compared to HHV-6A U37 with the control protein, suggesting that HHV-6A U37 interacts with HSF1. Furthermore, HSF1 inhibitor impaired HHV-6A-dependent stimulation of HSE-luc. These observations suggest that HHV-6A U37 specifically stimulates the HSE via the transcription factor HSF1. Taken together, this series of observations suggests that HHV-6A U37 interacts with and activates HSF1 to increase the expression of HSPs.

The role of heat shock signaling in HHV-6A replication.

JJhan cells were mock-infected or infected with HHV-6A U1102 for 72 h and then analyzed. Accumulation of Hsp90 was significantly enhanced in the HHV-6A infected cells, compared to the uninfected cells. Lastly, we tested different inhibitors of heat shock response on HHV-6A infected cells. Treatment of inhibitors of HSF1 or HSPs reduced the accumulation of viral proteins and viral yields.

3. Discussion

Our results emphasize the importance of heat shock signals in the viral life cycle. The region responsible for HSE activation in HHV-6A U37 was mapped to its N-terminus. However, this region in U37 homologues is less conserved, which may account for these differences. We propose a model in which HHV-6A U37 activates the heat shock response to support viral gene expression and replication. However, we cannot exclude the possibility that other viral proteins also contribute to this activation in infected cells, in addition to HHV-6A U37. Further studies are required to determine how and when HHV-6A U37 activates the HSE in infected cells. Our findings will provide important insights into novel interactions between herpesviruses and the heat shock response, and may suggest new targets for antiviral therapy against HHV-6A.

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

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論文題目 Title of Dissertation	ヒトヘルペスウイルス6A核タンパク質U37は、heat shock factor 1と相互作用し、heat shock応答を活性化する Human herpesvirus 6A nuclear matrix protein U37 interacts with heatshock transcription factor 1 and activates the heat shock response		
審査委員 Examiner	主査 Chief Examiner 副査 Vice-examiner 副査 Vice-examiner	鈴木 聡 勝二 郁夫 根本 秀樹	

（要旨は１，０００字～２，０００字程度）

（背景）

ヘルペスウイルスの新生ヌクレオカプシドは、内核膜を突き破って内核膜と外核膜の間の核周辺腔に出芽することにより、核外排出の際に一次エンベロープを獲得する。この過程は、核マトリックスタンパク質と核膜タンパク質からなる核脱出複合体（NEC）と呼ばれる保存されたウイルスヘテロ二量体複合体によって媒介される。核マトリックスタンパク質は、核脱出の際の重要な役割に加えて、NF-kBやIFN-γを含む細胞内シグナル伝達経路分子と相互作用し、ウイルスや細胞の遺伝子発現に影響を与えることが示されている。ヒトヘルペスウイルス6A（HHV-6A）U37遺伝子は、核マトリックスタンパク質をコードしているが、その役割はまだ解析されていなかった。

（結果）

本研究では、HHV-6A U37がヒートショックエレメント（HSE）プロモーターを活性化し、分子シャペロンHsp90の蓄積を誘導することを示した。機構的には、HHV-6A U37は熱ショック転写因子1（HSF1）と相互作用し、そのSer-326でのリン酸化を誘導した。また、HSF1、Hsp70またはHsp90を薬理学的に阻害すると、ウイルスタンパク質の蓄積とウイルス複製が減少することを報告した。このようにHHV-6A U37が熱ショック応答を活性化し、ウイルス遺伝子の発現と複製をサポートするというモデルを提唱した。

（重要性）

ヒトヘルペスウイルス6A（HHV-6A）は、βヘルペスウイルス亜科のロゼオロウイルス属に属するdsDNAウイルスである。HHV-6Aは神経炎症性疾患の患者からしばしば検出されるが、その病原的役割の解明が待たれている。熱ショック応答は、ホメオスタシスを破壊するようなストレス条件下での細胞の生存に重要である。

本研究結果では、HHV-6A U37がHSEプロモーターを活性化し、熱ショックタンパク質の蓄積を引き起こし、これらの熱ショック応答がウイルスの複製に重要であることを示した。

以上本研究は、宿主細胞のシグナル伝達における HHV-6A U37 の機能について新たな洞察を与え、HHV-6A の病原性と複製に関与する潜在的な細胞標的を同定するもので、非常に価値が高いことから、本研究者は、博士（医学）の学位を得る資格があると認める。