Human Herpesvirus 6A Tegument Protein U14 Induces NF-κB Signaling by Interacting with p65

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学 位 論 文 の 内 容 要 旨

Human Herpesvirus 6A Tegument Protein U14 Induces NF-κB Signaling by Interacting with p65

ヒトヘルペスウイルス 6A テグメントタンパク質 U14 は、
p65 と相互作用して NF-κb シグナルを誘導する

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1. Introduction

HHV-6A belonging to *Betaherpesvirinae* has frequently been reported to cause different neuroinflammatory diseases. The virus encoded tegument protein, U14 shares homology with other betaherpesvirues and is proven as an important factor for virus maturation. The activation of transcription factors NF-κB is recognized as a master-regulator in controlling multiple early cell-signaling events. The pathway activates upon signal-induced activation of IκB-kinase complex that causes the degradation of inhibitory protein IκB-α, subsequently, NF-κB dimeric subunit p65-p50 freely translocate from cytoplasm to nucleus and initiates the transcription of downstream genes that control different cellular behaviors. Besides controlling the expression of cellular genes, the pathway also reported to influence the replication of different viruses by either inhibiting or inducing the expression of virus genes. Considering the importance of U14 in HHV-6A replication and other important functions, in this present study, we focus on a novel function of this protein in NF-κB pathway.

2. Materials and Methods

To determine the role of U14 in NF-κB signaling, initially, we conducted a luciferase reporter assay in HEK293T cells by transfecting the cells with plasmids carrying U14 together with a set of firefly luciferase reporter plasmids harboring responsive element for NF-κB, IFN-β, ARE, and CRE. To confirm the specificity of U14 in such signaling, another round of luciferase reporter assay was performed with drug-induced inhibition or activation of NF-κB under U14 expression conditions. Soon after that, we performed subcellular fractionation experiments to determine whether U14 induced NF-κB subunit p65 to translocate into nucleus or not. Furthermore, we analyzed the expression of different downstream genes controlled by NF-κB pathway in either HEK293T cells exogenously expressing U14 or HHV-6A infected cell line. Moreover, we analyzed the physical interaction of U14 with NF-κB protein p65 by immunoprecipitation as well as immunofluorescence assay. Finally, we focused on the importance of this signaling in HHV-6A replication by analyzing the expression of virus protein and genome copy number in virus infected cells under drug induced inhibition of NF-κB transcription factors.

3. Results

HHV-6A U14 activates NF-κB pathway

Ectopic expression of HA-tagged U14 in HEK293T cells significantly stimulated the activity of NF-κB-luciferase but did not affect other promoters harboring INF-β, ARE or CRE-luc. This U14-dependent stimulation of NF-κB-luc activity was impaired followed by drug-induced inhibiton by interfering with p65 nuclear translocation, whereas the IKK inhibitor had no effect suggest an IKK-independent manner.

In addition, our subcellular fractionation analysis found that the amount of NF-κB subunit p65 in nucleus was increased in the presence of U14, indicating a prominent activation of NF-κB pathway

HHV-6A U14 increase the expression of NF-κB regulated gene:

Quantitative PCR (qPCR) revealed that transfection of cells with HA-U14 significantly increased transcription of *IL-6, IL-8*, and *MCP-1*, all of which are NF-κB-regulated genes other than HPRT1, a non-targeted NF-κB genes. Incubation with the NF-κB inhibitors SC75741 or QNZ for 60 min blocked the HHV-6A U14-dependent increases in gene expression. The similar phenomenon was also observed with HHV-6A infected cells.

Interaction of HHV-6A U14 with NF-κB regulatory proteins

The Coprecipitation experiments in cells transfected with Strep-tagged HHV-6A U14 (Strep-U14) or control plasmid found that the NF-κB component p65 was specifically coprecipitated with Strep-U14. Similarly, anti-HHV-6A U14 antibody coprecipitated the NF-κB component p65 from lysates of HHV-6A-infected cells but not from mock-infected cells. Further, we found by confocal imaging that transfected HA-U14 was colocalized with p65 in both cytoplasm and nucleus, however, the co-localization is more prominent in nucleus. In addition, HHV-6A infected cells also showed almost similar colocalization of U14 with p65. This result demonstrates that HHV-6A U14 can interact with NF-κB proteins and induce the expression of NF-κB-regulated genes in infected cells.

Importance of the NF-κB pathway in HHV-6A gene expression

Our earlier data showed that NF-κB promoted the expression of inflammatory cytokines in HHV-6A-infected cells, which possibly accelerates the pathology induced by HHV-6A infection. So, we then analyze the importance of this pathway in viral gene expression and replication upon infecting JJhan cells with HHV-6A U1102 for that were later subjected to treatment with NF-κB inhibitors SC75741 and QNZ. Immunofluorescence and immunoblotting analysis found that majority of infected cells that were not treated with the NF-κB inhibitor, expression of the immediate early protein IE2, as well as the late proteins U14 and gQ1, could be detected well. However, these proteins were barely expressed in infected cells that were treated with either SC75741 or QNZ. Moreover, qPCR analysis showed that both of the inhibitors significantly reduced the number of HHV-6A genome copy in the supernatant of the infected cells unlike non-treated cells, suggesting that NF-κB signaling is important for viral gene expression and progeny viral yields in JJhan cells.

4. Discussion

NF-κB is a crucial element of immunity associated with the regulation of antiviral response, however, pathogens can in some instances develop alternative mechanisms to "hijack" NF-κB-driven antagonism and exploit it for their benefit. We found that NF-κB-responsive promoters can be activated in the presence of HHV-6A U14. Also, transfection or viral infection led to an HHV-6A U14-dependent redistribution of the p65

subunit of NF-κB to the nucleus. Moreover, we showed that interleukin-6 (IL-6), IL-8, and monocyte chemoattractant protein 1 (MCP-1) transcripts were upregulated in the presence of HHV-6A U14 in an NF-κB-dependent manner. Since NF-κB signaling is also important for HHV-6A gene expression, our results suggest that HHV-6A U14 plays an important role in viral replication. The NF-κB inhibitors used in this study possibly impaired viral gene expression and replication through their effects on cell viability. Since, NF-κB is also important for HHV-6A gene expression and viral propagation at later times after infection, HHV-6A U14 might help to maintain the constitutive expression of immediate early and early genes and/or activate cellular genes responsible for viral replication through NF-κB signaling at later times after infection. To reveal the significance of the interaction between HHV-6A U14 and NF-κB, the p65 binding site on HHV-6A U14 must be determined in future studies. All in all, our findings provide important insight into the novel interaction between viruses and NF-κB signaling and might suggest new targets for antiviral therapy.

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 テグメントタンパク質 U14 は、p65 と相互作用して NF-κb シグナルを誘導する Human Herpesvirus 6A Tegument Protein U14 Induces NF-κB Signaling by Interacting with p65		
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（要旨は１，０００字～２，０００字程度）

【目的】

ウイルスに感染すると、宿主細胞は様々な免疫反応を引き起こし、ウイルスの伝搬や複製を制御する。たとえばNF-κBの活性化は、ウイルスの複製を調節する。

ベータヘルペスウイルス亜科に属するヒトヘルペスウイルス6A（HHV-6A）は、神経炎症性疾患の患者から頻繁に検出されるが、その病態への関与は明らかにされていない。

そこで本研究では、HHV-6A テグメント蛋白質U14による炎症やウイルス複製機構への関与を検討することとした。

【結果】

HHV-6A がコードする U14 タンパク質は、(1)NF-κB 複合体タンパク質である p65 と結合して相互作用することで NF-κB シグナルを活性化すること、(2)p65 の核内移行を誘導することにより、IL-6、IL-8、MCP1 の発現を増加させること、(3)NF-κBシグナルを阻害すると、ウイルスタンパク質の蓄積とウイルスゲノムのコピー数が減少するため、HHV-6Aの複製にはNF-κBシグナルの活性化が重要であること、などを見出した。

【結論】

以上より、HHV-6A感染によりNF-κB経路が活性化され、U14などの初期遺伝子産物を介してウイルスの効率的な複製と炎症性サイトカイン発現上昇に重要であることを明らかにした。

以上本研究は、宿主細胞のシグナル伝達におけるHHV-6A U14の機能に関する学術的な新しい発見のみならず、HHV-6Aの病因と複製に関与する治療標的の提示という臨床応用への可能性をも提示するもので、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。