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学位論文名	Intracellular Processing of Human Herpesvirus 6 Glycoproteins Q1 and Q2 into Tetrameric Complexes Expressed on the Viral Envelope （HHV-6 ウイルスエンベロープにおいて四量体を形成する糖タンパク gQ1、gQ2 の細胞内プロセッシング）
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論文内容の要旨

［目的］

Human herpesvirus 6 (HHV-6) is a betaherpesvirus related to human herpesvirus 7 (HHV-7) and human cytomegalovirus (HCMV) and is a human pathogen of emerging clinical significance. HHV-6 isolates can be categorized as two variants, A (HHV-6A) and B (HHV-6B). Herpesviruses encode a number of glycoproteins present in the envelope of the virion and play an important role in viral infection. Recently, we found that the HHV-6 gH-gL complex interacts with, gQ-80K. Furthermore, the gH-gL-gQ-80K complex of HHV-6A was identified as a viral ligand for human CD46, which is a cellular receptor of HHV-6. In this study, we focused on the further analysis of gQ gene products, which is unique to HHV-6 and HHV-7.

［方法ならびに成績］

Transcriptional analysis of the U100 (gQ) gene.

HSB-2 cells were infected with HHV-6A strain, GS or were mock infected ; poly (A)+RNA was extracted and was analyzed by Northern blotting and 5' RACE. The another small transcript in the gQ gene region, which encodes a protein of 214 a.a was found.

Characterization of U100 (gQ) gene products in HHV-6A (strain GS)-infected cells and purified virions.

The monoclonal antibodies (Mabs) for small ORF described above, were produced. By using the Mabs, the 37-kDa (gQ-37K) and 34-kDa (gQ-34K) forms were detected in HHV-6A infected cells by Western blotting. Thus, besides gQ-80K, the gQ gene encodes an additional product whose mature molecular weight is 37 kDa (gQ-37K),and which is derived from a different transcript. The gQ-34K was endo H and PNGaseF sensitive, however the gQ-37K was endo H resistant, indicaing that the gQ-34K contained immature high-mannose N-linked oligosaccharides, and the gQ-37K contained complex N-linked oligosaccharides. Therefore we designated gQ-80K as gQ1 and gQ-37K as gQ2. Furthermore, only gQ2-37K was incorporated into virions.

Immunoprecipitaion of strain GS-infected cells.

GS-infected HSB-2 cells lysates were immunoprecipitated with anti-gQ1 or anti-gQ2 by using S^{35} methionine. Pulse-chase experiment demonstrated that gQ2-34K associates with gQ1-74K within 30 min of the pulse period. After a 1-h chase, these precursor forms had associated with the gH-gL dimer.

To confirm the interaction of these proteins, the lysates of HHV-6A infected cells were immunoprecipitated with Mab for gQ2, gQ1 or gH, followed by immunoblotting with the anti-gQ1, anti-gQ2, or anti-gL Mab or the anti-gH Ab. Interestingly, an anti-gH Mab coimmunoprecipitated mainly gQ1-80K and gQ2-37K with little gQ1-74K or gQ2-34K, indicating that although gQ2-34K and gQ1-74K interact in the endoplasmic reticulum, the gH-gL-gQ1-80K-gQ2-37K heterotetrameric complex arises in the post-endoplasmic reticulum compartment. The mature complex is subsequently incorporated into viral particles.

［総括］

In this study, we found that a 37-kDa gQ glycoprotein, gQ-37K which is another gQ gene product, also associates with gH, gL, and gQ-80K to form a heterotetrameric complex on the viral envelope. Two kinds of gQ glycoproteins, gQ-80K and gQ-37K, were encoded by two distinct transcripts, one large transcript, whose products correspond to gQ-74K and gQ-80K, and the other, small transcript whose products are gQ-34K and gQ-37K. HHV-6 forms a tetrameric glycoprotein complex on the viral envelope. The tetrameric complex gH-gL-gQ1-gQ2 associates with human CD46, indicating that the association of gQ2-37K with gH-gL-gQ1-80K may be important for the binding of CD46 by the complex and in the subsequent increase of entry in the virus entry process or in the virus infection cycle.

論文審査の結果の要旨

Pilailuk Akkapaiboon は、ヒトヘルペスウイルス６（HHV-6）がコードする糖タンパクの解析を行ってきた。その中で、他のヘルペスウイルスには見いだされていない HHV-6 に特異的にコードされた glycoprotein Q（gQ）の感染細胞における動態に焦点をあて解析を行ってきた。解析を行う中、gQ はスプライスの違いにより、大きく分けて二種類のタンパクをコードすることが判明し、この二種類のタンパクは、本研究により、新たに gQ1 および gQ2 と名付けられた。gQ1 は、ウイルス感染細胞の中でウイルス糖タンパク gH/gL と複合体を形成することが報告されていたが、本研究で新たに同定された gQ2 もその複合体の一部であることが判明した。さらにこの gH/gL/gQ1/gQ2 複合体は、ゴルジ装置で形成され、その後ウイルス粒子中に取り込まれることが本研究により見いだされた。本研究は、ヘルペスウイルスがコードするエンベロープ糖タンパクの新たな概念を見いだしたものであり、学位に相当する研究成果であると考えられる。