【22】

氏　　名	Rizalinda Sjahril（リザリンダ　シャハリル）
博士の専攻分野の名称	博　士（医　学）
学 位 記 番 号	第　２２７３１　号
学位授与年月日	平成21年３月24日
学位授与の要件	学位規則第４条第１項該当 医学系研究科予防環境医学専攻
学 位 論 文 名	Relationship between U83 gene variation in human herpesvirus 6 and secretion of the U83 gene product （HHV-6 における U83 遺伝子の多様性と U83 遺伝子産物の分泌との関係）
論文審査委員	（主査） 教　授　杉本　　央 （副査） 教　授　生田　和良　　教　授　塩田　達雄

論 文 内 容 の 要 旨

〔 目　　的 〕

The human herpesvirus 6 (HHV6) has two variants, A and B. The differences between the two are based on a number of different properties, such as epidemiology, in vitro growth, reactivity to monoclonal antibodies, restriction endonuclease mapping, and nucleotide sequence. The complete DNA sequence of HHV-6B strain HST has been compared with that of HHV-6A. Marked differences were found in some of the deduced protein sequences. The U83 gene of HHV-6B strain HST encodes a chemokine that functions as a chemoattractant for monocytes. The U83 gene in HHV-6A, lacking signal peptide, most likely resulted in the inability to secrete the gene product. To determine the U83 gene deviation between variants A and B, or acute and latent infections, their gene sequences were compared and analyzed. In addition, we attempted to demonstrate the relationship between the functional differences of the U83 gene products and the DNA sequence.

〔 方法ならびに成績 〕

Thirty six isolates and 1 donor DNA sample of HHV-6 variants A and B that were sequenced were obtained from individuals diagnosed with exanthem subitum and from bone marrow transplant recipients. Each HHV-6 isolate was propagated in umbilical cord blood mononuclear cells (CBMC), in RPMI-10%FCS culture medium, and viral DNA was collected from the supernatant after approximately 50% of the cells showed cytopathic effect. The viral DNA was amplified by PCR, cloned into the pGEM-T Easy vector and sequenced. Analysis was performed using an ABI PRISM 3100 genetic analyzer. At least 25 clones from each isolate were sequenced. Comparisons were carried out using the sequences of HHV-6A (U1102), HHV-6B (Z29) and (HST). The U83 genes of all HHV-6A isolates did not have signal peptide sequence. The U83 genes had no methionine at the initiation site of the signal peptide except for U1102 strain and the HHV-6A DNA sample, in which the U83 chemokine was encoded in a different frame of the signal peptide sequence. Most of all variant B viruses contained the complete U83 gene, even though some were found as a minor component. The length of the signal peptide also varied in different isolates, ranging from 19 to 21 amino acids. The difference between viruses from transplant recipients and exanthem subitum was not significant. However, there was a higher frequency of viruses having a stop codon in its gene sequence among the reactivated viruses.

Next, expression of the U83 gene products of variant B, i.e. the wild type of HST (WT), a first methionine knock-out of HST (KO), the frame shift type of HST (FS), and variant A (GS) were compared by transfection of HeLa cells with GFP-U83 fusion plasmids. In HeLa cells the U83 gene products were localized to the cytosol. The mRNA amount in the transfected HeLa cells measured by real-time PCR was lowest in WT, whereas in FS, KO and GS were 1.4, 2.9 and 2.0 times greater than WT, respectively. The amount of intracellular fusion protein in WT was found lower than FS, KO, and GS. The highest secreted U83 gene product was in WT, lower in FS and were scarcely detected in the medium culture of KO and GS. These results indicated that the U83 gene product was secreted in strains containing a methionine in the initiation site of the signal peptide. The small amount of U83 gene product secreted by FS might be due to in-frame mRNA transcription by misreading of the thymidine cluster.

Subsequently we performed sequence analysis of the U83 cDNA, in order to exclude the possibility that variations were generated in the transfected HeLa cells. No variation was found among the mRNA of GS U83-gene-transfected cells, but there were in WT and FS U83-gene-transfected cells. The variation of mRNA was the same as that of viral DNA.

〔 総　　括 〕

U83 sequencing suggested that variant A was evolutionally divergent from variant B, and that variant B could be separated into two subgroups, an HST-Z29 type and another type with a shorter U83 signal peptide. U83 gene variations accumulated in variant A as well as in reactivated variant B after transplantation. None of variant A viruses encoded the signal peptide found in variant B, and consequently no mature U83 gene product was secreted when using a eukaryotic expression system. The HST-Z29 type of U83 gene product was secreted into the medium, partially secreted in the frame-shifted HST-Z29, but not secreted when the gene encoding the initial methionine of the signal peptide was deleted.

論文審査の結果の要旨

ヒトヘルペスウイルス６(HHV-6)には２種類のvariantが存在し、HST株のU83遺伝子産物は単球に対する誘因物質として機能する。本研究はHHV-6AとBに蓄積した遺伝子変異を多数のウイルスとDNAサンプルから解析した。HHV-6Aの配列はBとは異なり、易感染性宿主で再活性化されたHHV-6B中には変異が蓄積されていた。HHV-6Aウイルスにはシグナルペプチドがコードされていなかった。HHV-6Bにおける変異はシグナルペプチド領域のTクラスターの数の揺らぎに起因していると考えられた。培養細胞発現系において、HST型の遺伝子産物は培養液中に分泌されるが、HHV-6A型と最初のメチオニンを潰したHST型の遺伝子産物は分泌されなかった。さらに、フレイムのずれたタイプでは部分的に分泌され、これはmRNAのTの数の揺らぎに起因していることが示唆された。本研究は様々な変異に対してもケモカイン蛋白質を分泌することを可能たらしめる柔軟性をウイルスが有することを明らかにした、よって、学位に値するものと認める。