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学位論文名	Human herpesvirus 6 rep/U94 gene product has a single-stranded DNA binding activity （ヒトヘルペスウイルス6がコードするrep/U94遺伝子産物は、single-stranded DNA binding活性を有する）
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論文内容の要旨

【Objective】

Human herpesvirus6(HHV-6) was first isolated from peripheral blood of patients with lymphoproliferative disorders and classified into two variants:HHV-6A and HHV-6B. Both variants contain an ORF U94, which encodes a protein homologous to AAV-2 Rep.

AAV-2 Rep possesses several biological activities, all of which are required for DNA replication. AAV-2 preferentially integrates within a defined region of the cellular genome. HHV-6 was recently reported to integrate into the human genome as well. Conservation between the HHV-6 and AAV-2 may, therefore, mean that HHV-6 REP possesses a similar range of functions advantageous to the survival of HHV-6 within the host.

Therefore, in this study, we tried to analyze the function of HHV-6 rep gene in HHV-6-infected cells.

【Methods And results】

Expression of HHV-6 rep(REP) in REP-baculovirus-infected SF9 cells. The pFastBac recombinant virus containing REP was prepared and infected Sf9 cells. REP was detected at very high levels in both the nucleus and cytoplasm in Bac-REP-infected Sf9 cells by IFA and Western blotting.

Immunohistochemical analysis of HST-infected MT-4 cells. HST-infected MT4 cells were collected at 12, 24, 48 and 72 h post infection(PI). After staining and washing, signals were detected by confocal microscopy. REP was expressed at only low level in HHV-6-infected MT4 cells at 24 h PI and accumulated to higher levels by 72 h. REP staining was overlapped with DAPI staining, indicating that REP located in nucleus.

Western blot analysis. Cell lysates were prepared from mock and infected cells, and subjected to Western blot analysis with the anti-REP Mab. The antibody recognized a 56-kDa polypeptide in Bac-RBP-infected Sf9 cells, HHV-6B infected MT4 cells.

REP DNA-binding assay. The fusion proteins MBP-REP and MBP was applied to unmodified cellulose or single-stranded(ss) DNA columns and monitored an elution profile. And nuclear extracts of SupT 1 cells

were mixed with each protein and applied to the columns. The ssDNA-cellulose column retained MBP-REP, but did not retain MBP. And REP was detected in the fractions eluted with higher concentrations of NaCl when the nuclear extracts of SupT 1 were included than when REP was applied alone.

【Summary】

In this study, we produced HHV-6 REP antibody(anti-REP Mab). By using it, we found that REP expressed only at very low levels in the nucleus and cytoplasm of HST-infected MT4 cells 24 h PI and it was accumulated to higher levels within 72 h. The anti-REP Mab recognized only HHV-6B REP and reacted with the N-terminus of the REP protein. Furthermore, we reported that MBP-REP fusion protein possessed ssDNA-binding activity and that when MBP-REP was mixed with nuclear extracts of SupT 1 cells, the ssDNA binding capacity increased. The increased binding supports the idea that REP may interact with other cellular proteins that themselves bind DNA, aha that these interactions result in the strong and tight binding of ssDNA.

論文審査の結果の要旨

本研究はヒトヘルペスウイルス6（human herpesvirus 6；HHV-6）がコードしているREP蛋白の機能解析を調査した論文である。HHV-6は、突発性発疹の原因ウイルスであり、主にリンパ球で感染増殖することができる。ほとんどのヒトが幼少時に感染しており、宿主とともに潜伏感染していると考えられている。本研究では、HHV-6 REP蛋白は、リンパ球細胞において感染後、後期に出現し、核に局在することを明らかにした。その発現量は、感染細胞においてかなり少量であり、多量に発現することにより、ウイルス増殖に悪影響を及ぼす可能性が示唆された。さらに、Single-stranded DNAに非特異的に結合することができるが、double-stranded DNAには特異的にも非特異的にも結合できないことを発見した。これらの結果はウイルス感染状態においてREPが、DNA複製過程あるいはその後のウイルス増殖に役割を果たしている可能性を示すという新しい知見を提供し、学位の授与に値すると考えられる。