論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	Retno Rahayu
論文題名 Title	Localization of latency-associated nuclear antigen (LANA) on mitotic chromosomes (分裂染色体上における LANAの局在)

Introduction

Latency-associated nuclear antigen (LANA) has important roles for viral DNA replication and genome segregation. LANA's abilities to bind host chromosomes and KSHV terminal repeat sequence (TR) are important for the maintenance of viral genome copies during latency. To maintain viral genome copies in the growing infected cells, the viral genome must be replicated synchronously according to the cell cycle, and be segregated equally into two daughter cells. LANA has been reported to associate with centromere protein such as CENP-F and Bub1 during mitosis. These findings suggest that viral genome segregation should be assured by cellular mitotic checkpoint. However it is not well understood how LANA is distributed on the condensed chromosomes during mitosis. To further investigate the localization of LANA during mitosis, we independently examined the localization of LANA on chromosome spreads of KSHV infected cells (BCBL1) and analyzed using the super-high resolution laser confocal microscopy followed by a correlative fluorescence microscopy-electron microscopy (FM-EM) method.

Methods

KSHV-infected BC3 and BCBL1 cells, and KSHV-negative BJAB cells were arrested by 1 mg/ml colcemid for 4 hours, and then, the cells were released into the colcemid-free medium and collect cells at appropriate times. An indirect immunofluorescence assay (IFA) using an anti-LANA antibody and polyclonal anti-centromere antibodies (ACA) was subjected to the mitotic chromosome spreads of various stages of mitosis. The images were obtained using Carl Zeiss Elyra S1 super high-resolution microscope. For the FM-EM analysis FluoroNanogold™ conjugate were utilized to detect the signals with both confocal microscope and electron microscope

Results

LANA generally localized separately from the centromeres during mitosis (Prometa-, meta- and anaphase), just some were found to be side by side or in close proximity with centromeres (~5%). Ten to twenty percent of LANA dots were found at the cohesive sites between two sister chromatid and at the peri-telomeric region. The distribution of LANA did not change throughout the mitosis phase. Complete colocalization between LANA and centromeres were not observed throughout mitosis in this experiment. Our results suggested that LANA could occasionally associates with centromeres, and thus the association between LANA and centromeres during mitosis might not be essentials for KSHV genome segregation into two daughter cells

Conclusion

LANA is randomly localized on mitotic chromosomes. Our findings suggest that LANA should not associate with particular location on condensed chromosomes to secure equal segregation of KSHV genome, but association between LANA and various proteins that constitute the condensed chromosomes is necessary to increase the accuracy of viral genome segregation.

論文審査の結果の要旨及び担当者

（申請者氏名）RETNO RAHAYU

論文審査担当者	（職）	氏　名
主　査	大阪大学教授	仁田 亮次
副　査	大阪大学教授	塩田 達雄
副　査	大阪大学教授	藤堂 剛

論文審査の結果の要旨

In KSHV latent infection, the viral gene expression is limited and the copy number of viral genome remains constant. Latency-associated nuclear antigen LANA (LANA) has an essential role in maintaining the viral genome copy number in growing cells. To persist in a growing cells, LANA helps to segregate the KSHV genome equally into sister chromatids during mitosis. Some studies reported that LANA associated with host centromeric proteins such as CENP-F and Bub1 in KSHV infected PEL cell lines in order to achieve equal segregation of the KSHV genome during mitosis. This study implies that LANA localizes at the centromeres/peri-centromeres region of chromosome during mitosis to be surveyed by cellular mitotic checkpoint. LANA, however, seemed to be distributed randomly on the mitotic chromosomes of KSHV infected PEL cell lines by our observation. In order to get a better understanding about LANA distribution on mitotic chromosomes, We independently examined the localization of LANA on mitotic chromosomes during mitosis by using super-resolution laser confocal microscopy followed by a correlative fluorescence microscopy-electron microscopy (FM-EM) method to elucidate where LANA localized around the condensed chromosomes. Our results demonstrated that LANA randomly localized at various loci and was not concentrated around the specific regions such as centromeres/peri-centromeres, telomeres/peri-telomeres and sister chromatid cohesion sites. We did not observe any colocalization between LANA and centromeres throughout mitosis. The distribution of LANA on mitotic chromosomes did not change from prometaphase until anaphase. Based on our findings, we would propose a model of KSHV genome segregation during mitosis that KSHV genome replicated once per cell cycle together with host DNA during S phase with attaching a host chromosome. The sister chromatid then was separated during anaphase. Our findings suggest that association between LANA and centromeric proteins should not be essential for KSHV to achieve equal genome segregation. Various proteins constituting condensed chromosomes such as heterochromatin (Hp1α), telomeric proteins (TRF1, TRF2) and cohesion (SMC1, SMC3) might help to increase the accuracy of KSHV genome segregation.