氏　　名	Madiha (マディハ) Salah (サラ) Ibrahim (イブラヒム)
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学位論文名	Varied Persistent Life Cycles of Borna Disease Virus in a Human Oligodendroglioma Cell Line （ヒトオリゴデンドロサイト細胞株において見られたボルナ病ウイルスの採る異なるライフサイクルについて）
論文審査委員	（主査） 教　授　生田　和良 （副査） 教　授　塩田　達雄　　教　授　松浦　善治

論文内容の要旨

[Introduction]

BDV is a highly neurotropic virus that persists in the central nervous system (CNS) of infected animals as horses and sheep as well as several other vertebrate species causing progressive meningoencephalomyelitis. In tissue culture, cell-specific variations in the replication pattern of BDV affecting the viral production level as well as that of the antigens have been reported. In this study, we aimed to further characterize BDV replication in oligodendroctytes (OL), which are seldom used in such a purpose.

[Methods and Results]

Isolation of BDV-persistently infected cell clones. Cell free virus derived from OL/BDV (persistently infected with the human isolate of BDV ; HuP2br) was incubated with uninfected OL cells, followed by single cell cloning in 96-well plate. We obtained 30 cell clones each of a single-cell origin.

Classification of the cell clones by Immunofluorescence (IF). According to the IF percentage of positive cells that expressed BDV antigens, the cell clones were classified into three types : I (>20%), II (<20%), and III (0%). Representative cell clones from types I and II were picked up for further characterization.

Quantification of the BDV-p40 expression among the cell clones. By antigen-capture ELISA and Western blotting, the expression level of BDV-p40 among the cell clones was variable, consistent with the difference in the percentage of the BDV antigen expressing cells between types I and II detected by IF.

Quantification of the mRNA signals within the cell clones. By Northern blot analysis the expression of the 1.2- and 1.9-kb transcripts was detected with a p40 antisense riboprobe. Type II cell clones showed lower expression level of these transcripts than type I and the parental clone, consistent with the above results. Moreover, using the p40 sense riboprobe to detect the BDV genomic RNA, the expression level was also lower in type II than type I.

Detection of BDV RNA within the cells of types I and II cell clones. The cell population positive for the mRNA as well as the genomic RNA were quantified by in *situ* hybridization (ISH). Using p40 antisense riboprobe, the mRNA harboring cell percentages were consistent with the above result ; lower in type II than type I. The percentage of cells harboring the genomic RNA in type I was high as expected. On the other hand, the type II cell clones, which have a low percentage of antigen positive cells had more than two-three folds higher cell percentage harboring the genomic RNA than that expressing the protein or the mRNA. By recloning from type II, the same expression pattern was obtained again ; higher percentage of cells harboring the genomic RNA with lower percentage of cells having the protein and mRNA expression.

Enhancement of replication and transcription in type II cell clones.

(*a*) Serum starvation : following serum starvation, type II cell clones showed a gradual increase in protein positive cell number detected by IF.

(*b*) Nerve growth factor (NGF-β) treatment : by IF as well as ELISA, a gradual increase in the BDV-protein expressing cells in addition to increased intensity per cell was detected following NGF-β treatment.

Activation of MAP kinases in OL cells persistently infected with BDV by NGF-β. To examine whether an intrinsic cellular factor(s) could be involved in the activation of BDV in type II cell clones by NGF-β, we checked for the activation of the MAP kinases (ERK1/2). Before treatment with NGF-β, type I as well as the parental clone expressed the activated ERK 1/2. On the other hand, type II as well as OL (uninfected control), expressed the activated ERK 1/2 only after NGF-β treatment indicating a role for the MAP kinases in enhancement of BDV replication following NGF-β treatment.

[Conclusion]

Type II cell clones in which $<20\%$ of the population positive for BDV-specific proteins harbored the genomic RNA in about 40-60% of the cell population. Serum starvation as well as NGF potently enhanced BDV replication in type II as well as type I. Thus, we postulate that BDV established a restricted persistent infection in type II cell clones which could represent an in vitro state of latency for BDV in OL cells. Furthermore, the in vivo model with cell-free virus from type II cell clones may contribute to the understanding of the pathogenesis of BDV persistence or even latency in the non dividing neuronal cells in the CNS. Thus, BDV may be regarded as not always establishing a persistent infection with high levels of viral expression but may under certain conditions establish a latent infection with low levels of viral expression.

論文審査の結果の要旨

ボルナ病ウイルス（BDV）は、ウマやヒツジに脳脊髄炎を引き起こす一本鎖、マイナス鎖の非分節型（NNS）のRNA ウイルスで、Mononegavirales に属する。しかし、Mononegavirales に属する他のウイルスと異なり、核内で増殖する特徴を持っている。このウイルスは神経親和性が高く、感染動物の中枢神経系で持続感染する。BDV 持続感染成立は動物ばかりではなく、培養細胞においても認められる。

Madiha S. Ibrahim は、BDV に持続感染したヒトオリゴデンドロサイト由来細胞株から 30 個のクローン細胞を樹立した。BDV 抗原（p40 ヌクレオプロテインおよび p24 リン酸化蛋白）の発現程度から、Type I（$>20\%$）と Type II（$<20\%$）に型別した。in situ hybridizatrion（ISH）によるウイルス mRNA 解析によっても同程度の発現率であったことを確認している。一部の Type II クローンは、抗原や mRNA 発現率は低いが、genomic RNA 発現率が高く、再クローニング後も同様の傾向を示し、これらのクローンは、Nerve growth factor 添加によりウイルス抗原発現程度を顕著に亢進すること、この亢進には、MAP kinase の活性化と関連していることを証明している。このように、本研究で見出した知見は、BDV が一種の潜伏感染状態を培養細胞株で成立させることを示したものであり、学位の授与に値すると考えられる。