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学 位 論 文 要 旨

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題　　目：

Molecular biological studies on host cell responses involving rabies virus matrix protein

狂犬病ウイルスM蛋白質が関与する宿主細胞応答の分子生物学的研究

論文要旨：

Rabies is a fatal neurological disease caused by the rabies virus (RABV) that infects almost all mammals, including humans. There is no effective treatment after the onset of the disease, and approximately 59,000 human deaths occur annually worldwide. To develop more effective vaccines and new treatments after disease onset, accumulating basic information on the viral replication and virulence mechanisms is necessary.

In general, encephalitis-causing viruses induce severe inflammation and cell death in the host brain. However, inflammation, degeneration, and cell death are rarely observed in the brains of patients with rabies, suggesting that RABV has a mechanism to evade innate immunity and cell death. To investigate the molecular biological mechanism of RABV in evading innate immunity and suppressing cell death, I compared the Nishigahara strain, a highly pathogenic fixed RABV strain that can evade innate immunity and cell death, and the Ni-CE strain, a highly attenuated strain derived from the Nishigahara strain that strongly induces innate immunity and cell death.

In chapter I, I focus on stress granules (SGs), which have been recently reported to play an antiviral role. SGs are dynamic cellular structures that store untranslated mRNA and abnormal proteins when cells are subjected to stresses such as chemicals and heat shock. SGs have been recently reported to play an antiviral role by acting as a scaffold to activate retinoic acid-inducible gene I (RIG-I), a viral RNA sensor protein that promotes interferon (IFN) induction. However, the relationship between the immune evasion mechanisms of RABV and SGs remains unknown. Therefore, I investigated the difference in SG formation between the Nishigahara and Ni-CE strains. I found that Nishigahara-infected cells did not form SGs, whereas Ni-CE-infected cells formed numerous SGs, indicating that the avirulent Ni-CE stain, but not virulent Nishigahara, lose the ability to inhibit SG formation. Furthermore, I identified that RABV proteins and amino acids play key roles in SG formation by utilizing chimeric viruses and point mutation strains subject to gene substitution. The RABV genome encodes five structural proteins: nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and large protein (N, P, M, G, and L, respectively). I investigated SG formation in cells infected with Nishigahara chimeric strains, in which each gene of RABV proteins of the Nishigahara strain was replaced with that of the Ni-CE strain. I found that only the Ni(CEM) strain, the Nishigahara chimeric strain in which the gene of M protein was

replaced, induced a strong response similar to that of the Ni-CE strain. Because of two amino acid differences in the M protein at positions 29 and 95 between Nishigahara and Ni-CE strains, I investigated SG formation in cells infected with mutant strains, in which the amino acid at position 29 (M29) or 95 (M95) in the M protein was interchanged between the Nishigahara and Ni-CE strain [Ni(CEM29), Ni(CEM95), CE(NiM29), and CE(NiM95]. Nishigahara, Ni(CEM29), and CE(NiM95) strains, in which M95 is Val, inhibit SG formation, whereas Ni-CE, Ni(CEM95), and CE(NiM29) strains, in which M95 is Ala, did not inhibit SG formation, indicating that M95 plays a critical role in SG formation in the Ni-CE strain.

In chapter II, I elucidated the mechanism of SG formation related to M95. In general, entry of a virus into a cell can cause phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), and phosphorylated-eIF2α (p-eIF2α) activates SG formation in the cytoplasm. Among cellular kinases, protein kinase R (PKR) phosphorylates eIF2α. Therefore, I focused on the PKR activation and eIF2α phosphorylation in 293T cells infected with the Nishigahara, Ni-CE, Ni(CEM95), and CE(NiM95) strains. I demonstrated that Ni-CE and Ni(CEM95) strains induce SG formation via the PKR activation and eIF2α phosphorylation, whereas the Nishigahara and CE(NiM95) strains inhibit these processes. Furthermore, RIG-I accumulation in SGs and the initiation of IFN-β transcription occurred in cells infected with Ni-CE and Ni(CEM95) strains, suggesting that RIG-I utilized SGs as a scaffold to recognize the RABV genomes and promoted IFN-β transcription. In summary, SG formation induced by the Ni-CE strain occurs through PKR activation and eIF2α phosphorylation, resulting in the RIG-I accumulation for SGs and the initiation of IFN-β transcription.

In chapter III, I focused on the involvement of M95 in cell death and discussed its unknown mechanism. The Nishigahara and CE (NiM95) strains, in which M95 is Val, are unlikely to induce cell death, whereas the Ni-CE and Ni (CEM95) strains, in which M95 is Ala, dramatically induce cell death (Mita *et al*., *Virus Res*., 2008). This cell death has been predicted to be apoptotic, but this had not been verified. Therefore, I analyzed the cell death dynamics involving M95 and found that this cell death has apoptosis-related features: phosphatidylserine exposure, caspase-3 and caspase-7 activation, and TUNEL positivity. However, treatment with apoptosis inhibitors did not suppress cell death by the Ni-CE strain, suggesting that a new type of programmed cell death is involved.

In conclusion, I elucidated that the virulent fixed Nishigahara strain inhibits SG formation, whereas the Ni-CE strain, a non-lethal offshoot of the Nishigahara strain, does not, identifying that M95 plays a critical role in SG formation. Furthermore, RIG-I utilizes SGs as a scaffold to recognize the RABV genome, promoting IFN-β transcription. Moreover, I found that programmed cell death associated with M95 is not only involved in apoptosis but also in new types of cell death. Interestingly, M95 is involved in RABV pathogenesis (Ito *et al.*, *J Vet Med Sci.*, 2011), and there is a high possibility that M95-related pathogenesis is caused by the immune evasion of SG formation and programmed cell death.