論　文　内　容　の　要　旨

〔論文題名〕 Zinc-finger antiviral protein mediates retinoic acid inducible gene I-like receptor-independent antiviral response to murine leukemia virus
(ZAPはマウス白血病ウイルスに対するRLR非依存的な抗ウイルス応答を仲介する)

専攻名：　予防環境医学専攻　　氏名：　Lee Hanna (イ ハンナ)

〔目　的〕

Murine leukemia virus (MLV), a retrovirus belonging to the gammaretroviral genus of the family Retroviridae, is a causative agent of cancer in murine hosts. Although it is well known that Toll-like receptor 7 (TLR7) detects the genomic RNA of incoming MLV in endosomes and mediates the antiviral response, the RNA-sensing PRR that recognizes the MLV in the cytosol is not fully understood. RIG-like receptors (RLRs) might mediate the antiviral response to MLV after the viral RNA is detected, independently of type I IFN because RLRs stimulate not only IRF3/IRF7, but also other transcription factors, such as NF-κB and activator protein 1, which are responsible for the production of inflammatory cytokines and chemokines. Another candidate sensor is zinc-finger antiviral protein [ZAP, also called zinc finger CCCH-type, antiviral 1 (ZC3HAV1)]. ZAP was originally identified with an expression cloning method as one of the antiviral proteins directed against MLV. In this study we examined the roles of these two types of cytosolic RNA sensors and demonstrated the spatial regulation of the innate immune response directed against intracellular MLV.

〔方法ならびに成績〕

First, we used *RIG-I*$^{-/-}$*/MDA5*$^{-/-}$ and *IRF3*$^{-/-}$*/7*$^{-/-}$ mice to examine the involvement of RLRs in the antiviral response to MLV in mouse embryonic fibroblasts (MEFs). The results showed that MLV evades the RLR systems and does not induce the type I IFN response in MEFs. Next, we generated *Zc3hav1*$^{-/-}$ mice to examine whether endogenous ZAP controls the replication of MLV. The results showed that endogenous ZAP suppresses the replication of MLV in MEFs. Copy numbers of MLV genome were counted by quantitative PCR after isolation viral RNA from viral supernatant of infected or uninfected MEFs. In order to determine the localization of ZAP, we performed immunostaining assay. The results showed that ZAP located to the RNA granules where the exosome components and marker proteins for processing bodies assemble. Finally, we used fluorescence *in situ* hybridization (FISH) assay to clarify where MLV transcripts localize. The results showed that MLV transcripts mainly localized in the RNA granules after ectopic expression of ZAP. The results also showed that the CCCH-type zinc finger domains of ZAP, which are RNA-binding motifs, mediate its localization to RNA granules and MLV transcripts degradation by the exosome. Although ZAP was known as a regulator of RIG-I signaling in a human cell line, ZAP deficiency does not affect the RIG-I-dependent production of type I IFN in mouse cells.

〔総　括〕

We definitively demonstrate that ZAP induces the degradation of the MLV transcripts by the exosome, an RNA degradation system, on RNA granules. Thus, ZAP is a unique member of the cytosolic RNA-sensing PRR family that targets and eliminates intracellular RNA viruses independently of TLR and RLR family members.

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論文審査の結果の要旨及び担当者

（申請者氏名）Lee Hanna（イ　ハンナ）

	（職）	氏　名
論文審査担当者		
主　査	大阪大学教授	審良静男
副　査	大阪大学教授	竹田潔
副　査	大阪大学教授	山本雅裕

論文審査の結果の要旨

自然免疫機構は、RNAウイルスの特徴的なRNA構造をパターン認識受容体により感知してその排除を行う。しかしながら、レトロウイルスのRNAを認識するパターン認識受容体については不明な点が多い。Lee Hanna氏は、ガンマレトロウイルスであるマウス白血病ウイルス（MLV）に関する研究を行い、次の研究成果を上げている；(1) RNAウイルスに対する自然免疫応答に必須と考えられてきたRig-I様受容体は、MLVに対する自然免疫応答には関わっていない；(2) Zinc finger Antiviral Protein (ZAP)は、細胞内に侵入したMLVのRNAを感知してその排除を行う；(3) ZAPは、CCCH型-Zinc finger ドメインを介して、P-bodyのマーカーが陽性となるRNA顆粒に局在する；(4) ZAPは、RNA分解に関わるエキソソーム構成因子をRNA顆粒へ引き寄せることにより、MLV RNAの分解を誘導する。これらの発見はレトロウイルスに対する自然免疫応答の新たな機序を明らかにするものであり、学位の授与に値すると考えられる。