学位論文内容の要旨
（Summary of Dissertation）

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Mechanisms of NLRC5 nuclear import and retention for enhanced MHC class I transactivation
（NLRC5の核内輸送および保持によるMHCクラスI転写活性の増強メカニズム）

【Background and Objectives】Major histocompatibility complex (MHC) molecules are critical to present antigenic peptides to T cells to activate adaptive immunity. Recent research has identified NLRC5 as the protein responsible for MHC class I transactivation. NLRC5 is an IFN-γ-inducible gene and belongs to the NLR or nucleotide-binding domain (NBD), leucine-rich repeats (LRRs) family. Similar to other NLR proteins, NLRC5 has a tripartite domain structure; CARD, NACHT, and LRR. A CARD at its N-terminal contains bipartite nuclear localization signals (NLSs) which are required for the nuclear translocation. The centrally located NACHT contains a nucleotide-binding motif (Walker A) that is involved in the nuclear importation of NLRC5 and the transactivation of target genes. The longest LRR among NLR proteins is located at the C-terminal of NLRC5. Although most of the NLRs family proteins are known to be localized only in the cytoplasm, NLRC5 shuttles between the cytosol and the nucleus by its NLSs and it is relocalized into the nucleus by the IFN-γ stimultation. The importin α family is capable of recognizing the NLS of cargo proteins, importing them into the nucleus through the nuclear pore complex (NPC). However, the understanding of the mechanism of how importin takes NLRC5 to the nucleus is poor. In addition to nuclear import, proper regulation of export is important for optimal protein localization in the nucleus. CRM1, also known as exportin-1, is a nuclear export receptor that binds to leucine-rich nuclear export signals (NES) present in cargo proteins to facilitate their export from the nucleus. It has been reported that treatment with leptomycin B (LMB), a well-characterized inhibitor of CRM-1-mediated nuclear export, results in a significant increase in the nuclear localization of NLRC5, suggesting that NLRC5 export to the cytosol is a CRM1-dependent process. Histone acetyltransferases (HATs) are known as enzymes to induce histone acetylation and regulate gene expression. Moreover, some HATs have been reported to play an important role in regulating protein localization. In this study, we investigated the regulatory mechanisms underlying the reloalization of NLRC5 and showed the correlation between NLRC5 localization and its function. Specifically, we demonstrated that NLRC5 nuclear retention activated the MHC class I pathway. Furthermore, we identified that one of the HATs retained NLRC5 in the nucleus resulting in the increase of MHC-I transactivation.

【Methods】A NLRC5-deficient MCF7 cell line, a human breast cancer cell line, was generated by CRISPR-Cas9 system to study the function of NLRC5. Immunofluorescence analysis was performed to study the altered localization of NLRC5 between the cytosol and the nucleus by treatment with IFN-γ or LMB, or co-transfection with HATs. Subcellular protein fractionation was carried out to study the relocalization of NLRC5 from the cytosol to the nucleus by the treatment with or without IFN-γ in the presence of LMB. Mass spectrometry analysis was utilized to identify the NLRC5-specific binding proteins, which are confirmed by co-immunoprecipitation and western blotting. In addition, co-immunoprecipitation and western blotting were also performed to study the association of NLRC5 with KPNA6, CRM1 and GCN5, and the domain related with the NLRC5 export from the

nucleus. Luciferase reporter assay, quantitative reverse-transcription PCR analysis, and flow cytometry analysis were utilized to study the correlation of localization in NLRC5 and the MHC-I promoter activity, the expression at RNA level, and cell surface expression, respectively.

【Results】Immunofluorescence and subcellular protein fractionation analysis showed that IFN-γ treatment induced the nuclear accumulation of NLRC5, which was further enhanced by co-treatment with LMB, a CRM1 inhibitor. Luciferase reporter assay, quantitative reverse-transcription PCR analysis, and flow cytometry analysis showed that the IFN-γ-induced nuclear retention of NLRC5 enhanced by LMB treatment further elevated the promoter activity and the expression of MHC class I genes. Mass spectrometry analysis showed that NLRC5 specifically bound to one of the importin members, KPNA6, which was confirmed by co-immunoprecipitation. Co-immunoprecipitation demonstrated that NLS within CARD domain and Walker A motifs within NACHT domain are both essential for the recruitment of KPNA6. Additionally, we observed that the length of the LRR domain affected NLRC5 nuclear import, with shorter LRR domains showing more nuclear accumulation. GCN5, a member of the HATs family, was identified as a novel regulator that induced NLRC5 nuclear retention. Co-immunoprecipitation using co-transfection with NLRC5, CRM1 and GCN5 showed that GCN5 induced a decrease in the association of NLRC5 and CRM1 and an increase in the accumulation of NLRC5 in the nuclear, resulting in the activation of the MHC class I pathway.

【Discussion】Our previous work showed that the NLRC5 import to the nucleus is crucial for its function. In this study, our findings highlight a novel mechanism in the translocation of NLRC5, in which three domains of NLRC5 (CARD, NACHT and LRR) were required. Besides revealing the roles of the three domains in the nuclear localization, we identified KPNA6 and GCN5 as novel regulators to manipulate NLRC5 nuclear import and retention.

Some HATs are reported to alter the localization of proteins by binding in addition to regulating gene expression through histone acetylation. Although PCAF, one of the HATs, induces an increase in the accumulation of CIITA in the nucleus by acetylating CIITA protein, no difference in the localization of NLRC5 was observed by PCAF overexpression. Instead, another member of HATs, GCN5, altered the relocalization of NLRC5. A study using yeast reports that GCN5 interrupts the interaction of proteins to inhibit gene transcription. Similarly, in our study, the interaction between NLRC5 and CRM1 was inhibited by GCN5 resulting in inducing the increase in accumulation of NLRC5 in the nucleus. These results suggest that GCN5 was associated with the relocalization of NLRC5 in the nucleus, different from CIITA.

IFN-γ stimuli induces the import and retention of NLRC5 in the nucleus. However, no increase in the expression levels of NLRC5 relocalization associated protein, KPNA6 and GCN5, was observed. Clarifying the mechanism of the link between IFN-γ stimuli and the function of these proteins is required to better understand IFN-γ induced MHC-I transactivation via NLRC5. These results provide support for the targeting therapies that alter the present mechanisms to activate MHC-I transcription and elevate MHC-I expression.

【Conclusion】In conclusion, our findings uncovered the molecular mechanisms underlying the nuclear import and retention of NLRC5. The nuclear retention of NLRC5 enhanced the expression of MHC class I genes, suggesting that the intervention of the shuttling mechanism of NLRC5 may serve as a potential therapeutic target for virus infections and cancers.