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学位論文題目	Elucidation of leukemia-associated nucleoporin fusion genes' effects on the nuclear pore complexes and nuclear-cytoplasmic transport （白血病で見られる Nup 融合タンパク質が核膜孔複合体と核ー細胞質間物質輸送に与える影響の解明）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author describes impact of leukemia-associated fusions, NUP98- and NUP214-fusion genes, on intracellular distribution and integrity of nuclear pore complex (NPC) components. NPCs are comprised of approximately 30 nucleoporins (Nups), among which NUP98 and NUP214 genes are fused to partner genes by chromosomal translocation in hematopoietic malignancies. The author characterized SET-Nup214, DEK-Nup214, Nup98-DDX10 and Nup98-HOXA9 fusion gene products in leukemia-derived cell-lines, or using overexpression system in HeLa cells. Immunofluorescence microscopy and NPC immunoprecipitation assays consistently supported the effects of NUP-fusions in altered distribution of NPC components. The author also examined localization and function of exportin proteins and demonstrated mislocalization of exportin 4, 6 and 7 by SET-Nup214 fusion. With these observations, the author proposed that chromosomal translocations involving NUP98 and NUP214 induce cell malignancy in leukemia by impairing localization and expression of NPC components and affecting nuclear-cytoplasmic transport of critical factors for oncogenesis. The content is summarized as follows:

（目的　Purpose）

The purpose of this research is to examine impact of leukemia-associated NUP-fusion genes on composition, intracellular localization and function of NPC components and exportins.

（対象と方法　Materials and Methods）

To characterize impact of NUP-fusion gene products on NPC components, the author used T-cell acute lymphoblastic leukemia cells LOUCY which carry naturally-occurred SET-Nup214 fusion, and acute myeloid leukemia cells FKH1 which carry DEK-Nup214 fusion. Full-length Nup214 and SET-Nup214 and DEK-Nup214 fusions, as well as full-length Nup98, Nup98-DDX10 and Nup98-HOXA9 expression constructs were produced and used to assess the function of fusion proteins in HeLa cell model. Localization of NPC components was examined by immunofluorescence microscopy. Composition of NPCs was examined by immuno-purification of NPCs and Western blot. Stability of NPC components was examined by cycloheximide treatment to observe protein degradation in the cells.

To characterize impact on exportin proteins, the author expressed Nup214- and Nup98-fusions in HeLa cells. Intracellular localization of exportin proteins was examined by immunofluorescent microscopy.

（結果 Results）

The author showed that Nup62 and Nup88, representative NPC components, were differentially distributed in cell-line-specific manners. LOUCY showed nuclear dot-like appearance and FKH1 showed intra-nuclear distribution of Nup62 and Nup98. These observations were also supported by quantification of nuclear intensity. Differential appearance of intranuclear NPC components were further confirmed by expression of SET-Nup214 and DEK-Nup214 expression by transfection experiments in HeLa cells. The author examined impact of Nup98-DDX10 and Nup98-HOXA9 expression. Using additional NPC components, Nup88, Nup153 and POM121, nuclear accumulation of multiple NPC components induced by Nup-fusion protein was confirmed.

The author further supported mislocalization of NPC components by showing physical interaction of SET-Nup214 and Nup98-HOXA9 with endogenous NPC components by immunopurification followed by Western blot in HEK293T cells. By mapping regions within Nup214 and Nup98 using deletion constricts, the author showed that mislocalization of NPC components by Nup-fusions depended on C-terminus region (1057-2090) or Nup214 and N-terminus region (1-469) of Nup98 respectively. By siRNA-based depletion of Nup98 and cycloheximide-treatment assays, the author showed that stability of SET- or DEK-Nup214 fusion and endogenous Nup214 proteins was maintained by Nup98.

Finally, the author examined localization of exportins, XPO1, XPO2, XPO3, XPO4, XPO5, XPO6 and XPO7 in the presence of SET-Nup214, DEK-Nup214, Nup98-DDX10 and Nup98-HOXA9, and showed that leukemia-associated Nup-fusions affected nuclear distribution of multiple exportins.

（考察 Discussion）

The author evaluated impact of leukemia-associated NUP98- and NUP214-fusion genes by examining distribution of NPC components and exportins as well as physical association among NPC components and protein stability. The results supported the author’s model that expression of Nup214 and Nup98-fusion proteins and reduction of endogenous full-length Nup214 and Nup98 affect integrity and localization of NPCs. Impaired NPC function could cause nuclear-cytoplasmic transport of factors which are relevant to oncogenesis.

審査の結果の要旨
Abstract of assessment result

（批評 General Comments）

The applicant systematically examined cellular localization and physical association of NPC components and exportins under NUP-fusion expression and depletion in leukemia cell-lines and in transfection models. Based on these results, the applicant highlighted that NPCs and exportin are controlled by the interaction among multiple components and leukemia-associated NUP-fusions may contribute cellular malignancy in leukemia by misregulating

critical targets of nuclear-cytoplasmic transportation.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on June 2, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.