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学　位　論　文　概　要
Dissertation Summary

学位請求論文（Dissertation）
題名（Title）　***The role of Nucleoporin Rae1 in cell fate determination***
（邦訳又は英訳）（Title in Japanese or in English）　細胞の運命制御における Rae1 の機能解析

専攻（Division）: Natural System
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学位論文概要（Dissertation Summary）

Nuclear pore complexes (NPCs), alongside as having the main function as nucleocytoplasmic trafficking, have also implicated in numerous basic cellular processes including mitosis, differentiation, chromatin organization, epigenetic regulation and so forth, which correlated with many diseases. It is the largest protein complexes in eukaryotic cells (~60 MDa in yeast and 90–120 MDa in humans) which are composed of ~30 types of nucleoporins (Nups).

Here, we concentrated studying one of the nups of NPC called Rae1 with “The role of Nucleoporin Rae1 in cell fate determination” as our main title, and divided the study into three chapters. They are: 1.) The study of Rae1 in cancer (preliminary work of Rae1-Breast cancer), 2.) The study of Rae1 in viral disease (Overexpression of SARS-CoV-2 protein ORF6 dislocates Rae1 and Nup98 from the nuclear pore complex), and 3.) Rae1 study in the future (Label-free tomographic imaging of nanodiamonds in living cells).

CHAPTER I. The preliminary study of the role of Rae1 in Breast cancer

Regarding to the study of Rae1 in cancer, previously, we and others showed that nucleoporin Rae1 is a binding platform for NuMA, cohesin subunits SMC1 to prevent aneuploidy during mitosis. Bioinformatic data analysis from The Cancer Genome Atlas (TCGA) showing that Rae1 is extensively amplified and overexpressed in several solid cancers including colon and breast cancer respectively. Using data from METABRIC & TCGA (Figure1), we found that amplification and gain was the first and second common abnormalities, suggesting breast cancers prone to show over-expression of Rae1

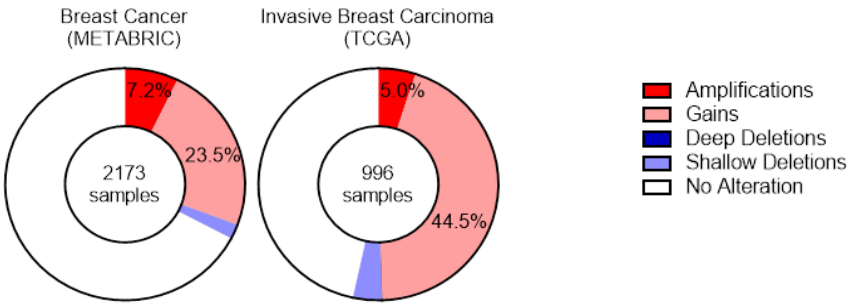

Figure 1. METABRIK & TCGA bioinformatic data

Consistent with in-silico data, the expression levels of Rae1 in MCF7 in our immunoblotting was elavated in MCF7 comparing to that in MCF10A (Figure 2.A). Moreover, from the immunostaining Rae1 in MCF7 cells localized in both nuclear membrane and nucleoplasm while the localization of Rae1 was mainly in the nuclear membrane in MCF10A, suggesting the cancer specific of Rae1 inside nucloplasm (Figure 2.B).

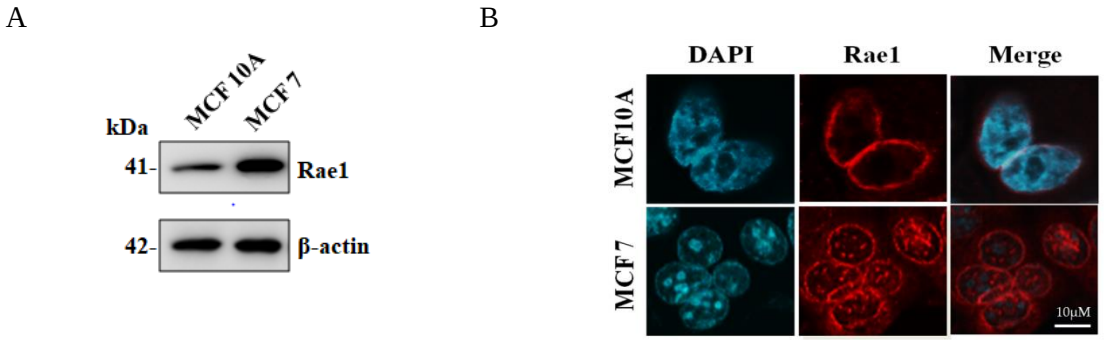

Figure 2. Rae1 expression in MCF10A vs MCF7. A. Immunoblott analysis. B. Immunofluorescence-confocal microscopy images.

Here, we first performed transcriptomic and proteomic study of Rae1 in MCF7 breast cancer cells to provide in depth-understanding about the global change of differentially expressed genes. Since Rae1 is localized in nucleoplasm, we hypothesized that Rae1 might be involved in transcriptional or epigenetic function, and are inspired to see Rae1 binding partners in breast cancer cells. Therefore, we utilized mass spectrometer (MS) to obtain peptide mass profiles from silver-stained protein in MCF7, where several protein interactors of Rae1 (Figure 3) were selected based on the peptides that give high peptide spectrum match (PSM) score.

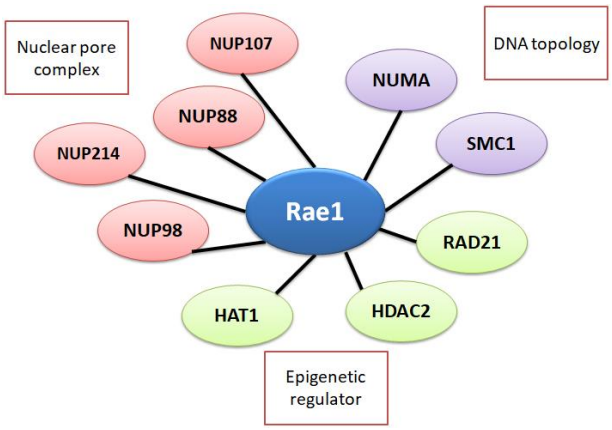

Figure 3. Protein partners of Rae1 selected from Mass Spectrometry

From the Phenotypic assay, our work suggested that Rae1 might be important to overcome susceptibility of apoptosis in MCF7 as depletion of Rae1 lead to the increasement of apoptotic marker under the anoikis condition. Taken the phenotypic assay together with proteomic data, we found that upregulated Rae1 is functionally involved in preventing anoikis, which is caused by novel role of Rae1 in nucleoplasm related to epigenetic regulation.

Chapter II. Overexpression of SARS-CoV-2 protein ORF6 dislocates Rae1 and Nup98 from the nuclear pore complex

In the second study we inspired to gain knowledge relevant to nucleoporin in correlation with viral disease. As the novel human betacoronavirus SARS-CoV-2 has caused an unprecedented pandemic in the 21st century. Several studies have revealed interactions between SARS-CoV-2 viral proteins and host nucleoporins, yet their functions are largely unknown.

Here, we demonstrate that the open-reading frame 6 (ORF6) of SARS-CoV-2 can directly manipulate

localization and functions of nucleoporins. Confocal microscopy was used to look at Rae1 localization in ORF6-overexpressing cells. ORF6 overexpression caused Rae1 to be displaced from the nuclear membrane to the cytoplasm in HEK293T cells and the human lung cancer cell line PC9, as seen in Figures 4.

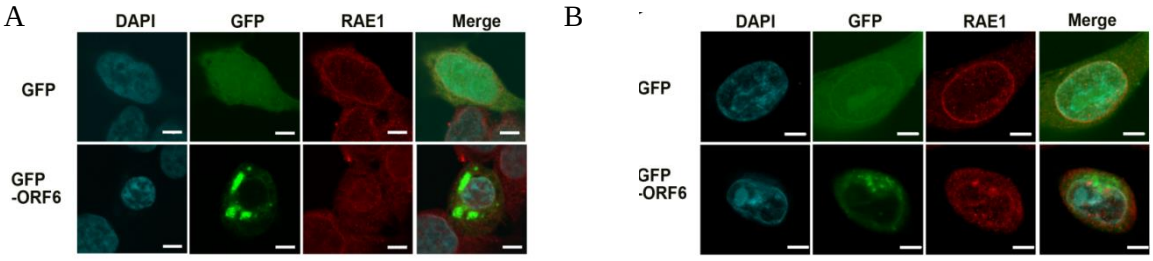

Figure 4. Confocal imaging of GFP and Rae1 localization in HEK293T (A) and PC9

We then used coimmunofluorescence labeling to look at the Rae1 binding partner Nup98. In comparison to GFP vector control cells, GFP-ORF6 overexpression caused Nup98 to be displaced from the nuclear rim to the cytoplasm in HEK293T cells and PC9 cells (figure not shown here). We also found that ORF6 expression alone is enough to disrupt the nucleocytoplasmic mRNA transport factors Rae1 and Nup98, resulting in mislocalization of the host endogenous mRNA export machinery and, as a result, a reduction in nuclear size.

We also looked into hnRNPA1 expression and localization as hnRNPA1 is normally associated to RNAs in the nucleus, as well as some cytoplasmic mRNAs, throughout their processing. According to our findings, overexpression of SARS-CoV-2 ORF6 alone did not shift hnRNPA1 to the cytoplasm,. In GFP-ORF6-transfected cells, the density of hnRNPA1 was dramatically enhanced and consistently remained inside the nuclear area. This finding also suggests that ORF6 overexpression caused hnRNPA1 to accumulate in the nucleus. Moreover, if our hypothesis is correct, overexpression of FLAG-Rae1 and GFP-ORF6 would partially relieve hnRNPA1 stacking through sequestration of endogenous nuclear hnRNPA1. Consistent with our prediction, we found that hnRNPA1 nuclear accumulation was partially relieved by hnRNPA1 nuclear stacking.

Therefore, as a conclusion, the Orf6 of SARS-CoV-2 has the ability to displace the Rae1 AND Nup98 complex from its main origin, resulting the disturbance of nucleocytoplasmic trafficking and evntually the accumulation of mRNAtransporters like hnRNPA1

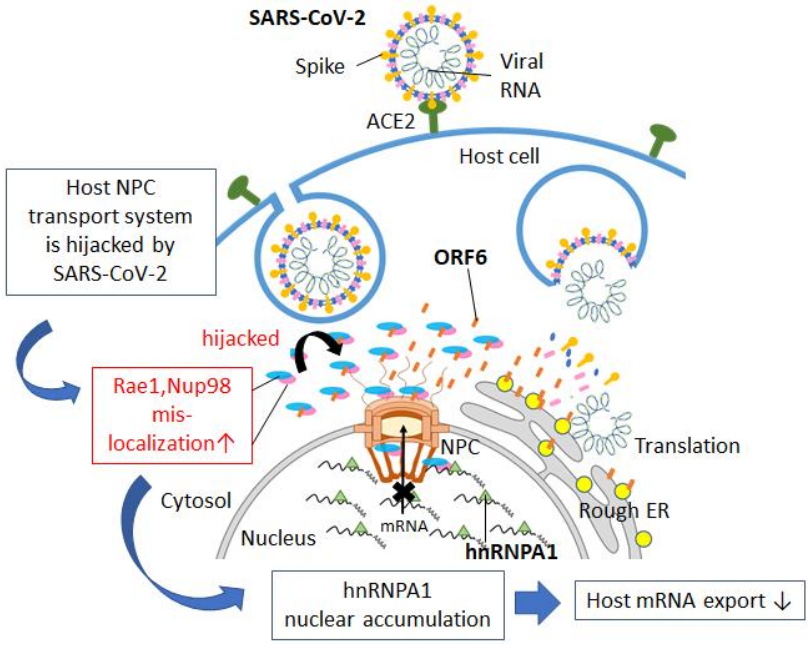

Figure 5. The pathogenesis of SARS-CoV-2 in association with Nucleocytoplasmic trafficking.

Chapter III. Label-free tomographic imaging of nanodiamonds in living cells (A new promising method to visualize tagged Rae1 in near future)

Given the huge impact of Rae1 in our laboratory's long work history, it is reasonable to consider the new, fast and reliable system dedicating for better labeling and imaging of this protein inside living cells. Nanodiamonds (NDs) have acknowledged growing attention due to their decent biocompatibility and photostability with its application as a molecular label is actually not a new phenomenon. However, despite its extensive utilization, major drawback such as phototoxicity from laser irradiation to detect the fluorescence signal still limits this long-time tracking of nanodiamonds in living cells. Other researcher of our laboratory in collaboration with Kyoto University has succesfully detect Rae1 in a conjugation form with Nanodiamonds (DNDs-HPG-Rae1) using high speed AFM (unpublished data). Prior to achieve future study of new Rae1 visualization method inside living cell facilitated by NDs, here, we performed novel quantitative morphological and biophysical celullar analysis by optical diffraction tomography (ODT) using various sizes and introduction methods of NDs. ODT is an inexpensive and noninvasive microscopy technique, which images cells and subcellular structures as a function of their refractive index (RI); hence, can reduce phototoxicity.

In this sudy, the very high RI of NDs in HeLa cells can be clearly discriminated from cellular structures. Aggregation and deaggregation of internalized NDs can be detected via changes in the RI distribution of the entire cell and the prevention of in-cell particle aggregation was observed through polyglycerol coating of NDs. In endocytosis method, optical diffraction tomography shows deaggregation of NDs after a prolonged incubation time. Together, these findings implicate that RI measurements are a favorable tool to track NDs, without a fluorescent label, inside living cells. This could be useful to study real time metabolic activities in living cells using very weak laser irradiation. Also, as our data indicated no significant changes of viability of cells receiving NDs treatment, we hopefully can continue to bioconjugate Rae1 into NDs and successfully tracked it inside the living cells by ODT in the near future. Figure 6 below summarized the advantage of new imaging NDs method using ODT system.

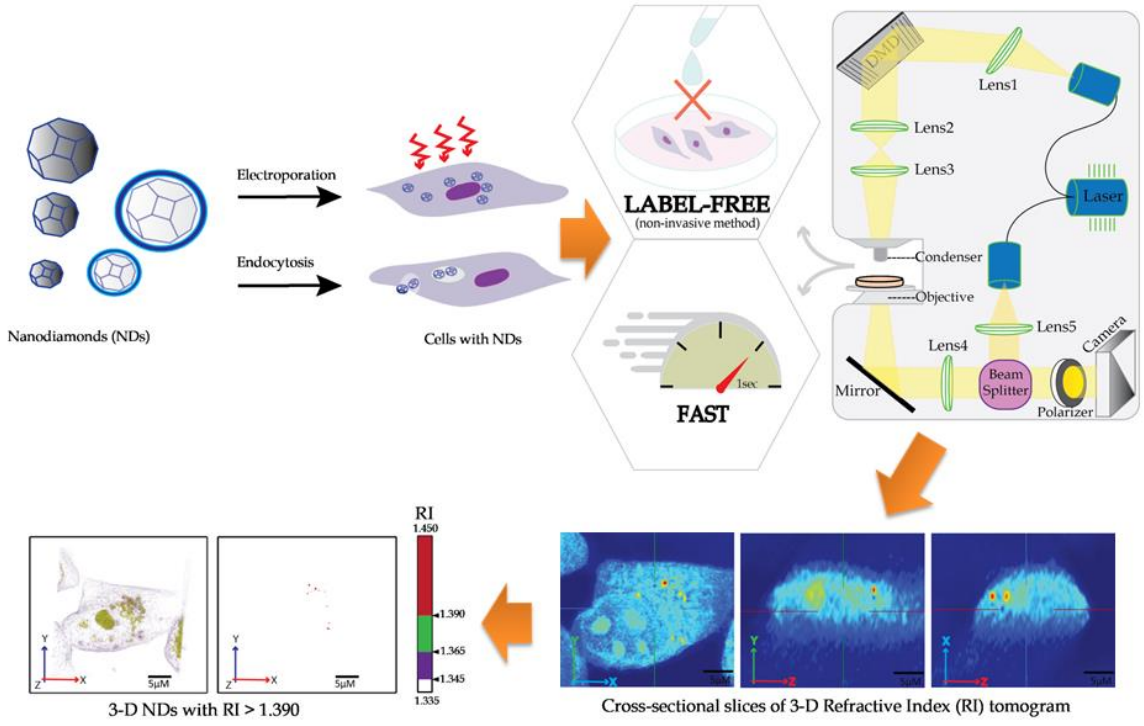

Figure 6**.** The principle and advantage of ODT to visualize NDs in a living cell