研究題目　Proteomics analysis of CSR specific recombination complex in B cell

研究組織

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【1】研究の概要

[1-1]本研究の目的・概要

Activation-Induced Cytidine Deaminase (AID) is the key enzyme for immune diversification at IgH locus. Specifically, In B cells it goes through two major complex steps i.e. DNA break and recombination. To understand more precisely the recombination step we conduct to major studies:

(A) Identification of RNA/DNA editing protein complex that associates with AID's function specific hnRNP co-factor.
(B) Identification of Ig locus specific chromatin protein complex important for AID induced class switch recombination or genomic instability.

[1-2]研究の方法・経過

The same mutants of hnRNPL were used as described in our previous report (Fig. 1). The CSR efficiency of various mutants was examined by monitoring IgM to IgA switching efficiency in a mouse B cell line. WT and mutant (Mut-1, Mut-2) proteins were also examined for their interaction or complex formation with AID in 293T and B cells. Since hnRNPL was tagged with FLAG epitope, we also performed anti-FLAG immunoprecipitation (IP) of WT and CSR defective hnRNPL mutants. The gel-free approach was taken this time to minimum sample loss and more precise analysis. IPed proteins samples conjugated with Ab-beads were directly sent for mass analysis as suggested by Prof. H. Kosako's laboratory at Tokushima University.

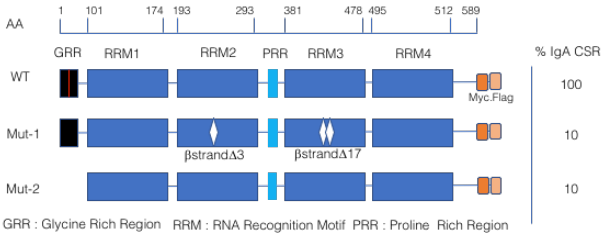

Figure 1. The schematic view shows the domain structure of wild type and two critical hnRNPL mutants. IgM to IgA switching efficiency was examined, which indicates that both are defective in CSR.

【2】研究成果

[2-1]本共同研究で明らかになった研究成果

Many promising candidates missing in mutants (Mut-1/Mut-2) appears from mass data. We specifically focused on RNA binding protein as AID interacts with hnRNPL in RNA dependent manner. After knockdown each RBPs we found all the candidates appears to be down and important for CSR (Fig. 2). It would be great finding as it suggests that hnRNPL has many independent complex which are involved in the CSR. Further confirmation or experiment need to be required to prove the complex specific function of CSR.

[2-2]本共同研究による波及効果及び今後の発展性

It is very interesting we found some new

candidates (CSTF2, TNRC6A and Spindilin etc.) which are playing important role in CSR. As we thought, hnRNPL interacts with many proteins together with AID make higher ordered structure for DNA repair in CSR.

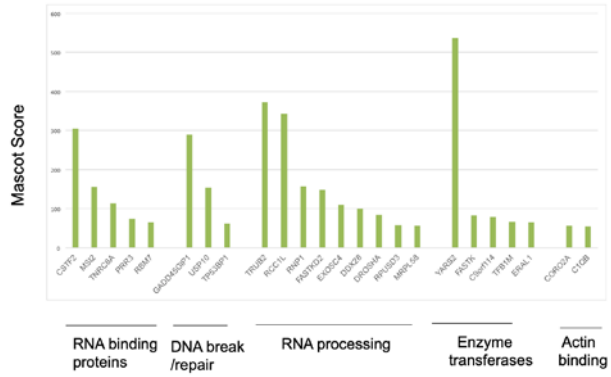

Figure 2. The missing proteins in the Mut-1 mutant but present in WT-hnRNPL.

【3】主な発表論文等

[3-1]論文発表
なし

[3-2]学会発表
なし

[3-3]成果資料等
なし

【4】今後の課題等

今後の課題、その他等

The detailed mechanisms need to be studied for CSR specific DNA repair together with hnRNPL and found proteins.