【Grant-in-Aid for Scientific Research (S)】

Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Regulatory Mechanism of Immunoglobulin Gene Diversification and Genome Instability by RNA-Editing Catalyzed by Activation-Induced Cytidine Deaminase (AID)

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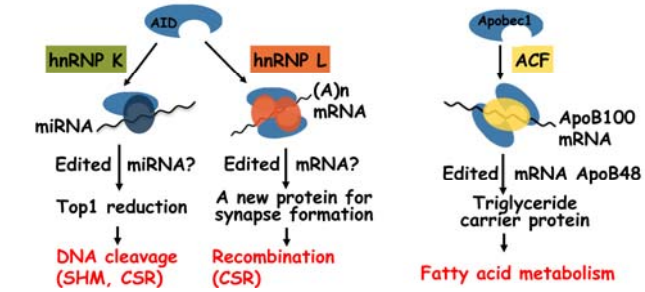
Research Project Number : 15H05784 Researcher Number : 80090504
Research Area : Medicine, Dentistry and Pharmacy
Keyword : DNA breaks, Recombination, Aquired immunity, Immunological memory

【Purpose and Background of the Research】

AID is the central and critical enzyme in generation of immunoglobulin gene (Ig) diversity and immunological memory which determines the efficacy of vaccination. AID employs Topoisomerase 1 (Top1) for DNA cleavage in class switch recombination (CSR) and somatic hypermutation (SHM) and induces genomic instability by causing aberrant DNA cleavage. This project aims to clarify the mechanism for Top1 translation regulation by RNA editing of the putative miRNA precursor(s) with AID and its cofactor hnRNP K. The target specificity of Top1 should be also elucidated. In addition we will identify the mRNA edited by AID and hnRNP L (the other cofactor) and characterize the function of a novel translated protein(s) in the repair step of CSR. AID is involved in metabolic pathogenesis through the regulation of intestinal flora by production of high affinity antibodies. In addition, mutation of Top1 processing enzymes trigger neuronal diseases. Therefore this project contributes to not only understanding the basic mechanism of acquired immunity, but also in metabolic deregulation by immune deficiency and transcription-dependent genome instability.

Possible Model:



【Research Methods】

The miRNA precursor(s) will be isolated by serial immunoprecipitation (IP) with AID and hnRNP K. Sequencing of these precursor(s) will unravel the exact base position edited by AID. The genome-wide analysis of non-B DNA structure by psoralen binding method and ChIP seq analysis detecting the accumulation of H3K4me3, Top1 and FACT complex will reveal the mechanism specifying the targets of AID-induced DNA breaks. iChIP methods using the cell line which has the lexA binding sequence in the S region will reveal proteins in the cleavage site-specific complex. The analysis of mRNAs binding to the complex of hnRNP L and AID will identify the proteins essential for DNA repair and recombination after DNA breaks. The function of these novel proteins will be associated with Brd4 which makes synapsis between distant two DNA break loci. Top1 covalently bound to the 3' end of DNA should be processed for efficient repair. The resection mechanism of Top1 from 3' end of DNA will be elucidated.

【Expected Research Achievements and Scientific Significance】

The elucidated mechanism of acquired immunity will be applied for the strategy of vaccine development. This project will clarify the function of hnRNP family proteins, which are largely unknown so far and contribute to the understanding of tumorigenesis by AID. This project will give the clear-cut view for pathogenesis of transcription-coupled genomic instability caused by Top1 deregulation, such as neuronal diseases and cancer. Identification of hypomorphic mutations of Top1, hnRNP K and hnRNP L in human will allow to assess a risk for polygenic diseases such as cancer or immunodeficiency.

【Publications Relevant to the Project】

Hu, W. at a ***Proc. Natl. Acad. Sci. USA*** 112 5791-5796 (2015). Stanlie, A. at al. ***Mol.Cell*** 55, 97–110 (2014). Kobayashi, M. et al. ***Proc. Natl. Acad. Sci. USA*** 108, 19305-19310 (2011). Kobayashi, M. et al. ***Proc. Natl. Acad. Sci. USA*** 106, 22375-22380 (2009). Muramatsu, M. at al. ***Cell*** 102 553-563 (2000).

【Term of Project】 FY2015-2018

【Budget Allocation】 153,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www2.mfour.med.kyoto-u.ac.jp/