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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Role of R-loop in antibody class switching and B cell lymphomagenesis

研究課題名（英文）ole of R-loop in antibody class switching and B cell lymphomagenesis

研究代表者
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研究成果の概要（和文）：ゲノムの完全性を維持することは、B細胞（免疫細胞の一種）におけるクラススイッチ組換えの過程で重要です。本研究では、HNRNPUというRNAを結合するタンパク質が、DNA修復と遺伝子の安定化に関わり、がんの原因となる染色体異常を防ぐ役割があることを発見しました。この成果は今後、治療法の研究にも役立つ可能性があります。

研究成果の学術的意義や社会的意義
The novel findings regarding the regulatory roles of HNRNPU and MED12 in recombination in B cells offer fresh perspectives on immune system functionality and linked genomic instability. These insights hold promise for the development of innovative diagnostic and therapeutic approaches in the future.

研究成果の概要（英文）：During class switching in mature B cells, maintaining genomic integrity is essential due to DNA breaks and rearrangements in the antibody gene locus. This study reveals that heterogeneous ribonucleoprotein HNRNPU is pivotal in regulating R-loop dynamics and DNA repair, while MED12 controls DNA breakage and locus-specific conformation. HNRNPU interacts with DNA repair factors and binds to the Ig locus's G4 RNA/DNA structures. Loss of HNRNPU disrupts DNA repair, leading to R-loop imbalances and hindering antibody class switch processes. Conversely, MED12 loss inhibits recombination by impairing AID-induced DNA breaks and S-S synapsis, which is crucial for bringing recombining loci into proximity. Thus, HNRNPU and MED12 are critical in coordinating immune responses and maintaining genomic stability.

研究分野：Molecular Immunology

キーワード：CSR　AID　HNRNPU　DNA Repair　NHEJ　MED12

1.研究開始当初の背景

Within the complex landscape of antibody gene diversification, antigen-activated B cells undergo somatic hypermutation (SHM) and class switch recombination (CSR), processes essential for generating high-affinity antibodies with diverse effector functions. These processes are orchestrated by Activation-Induced Cytidine Deaminase (AID), an enzyme crucial for introducing DNA breaks within the Immunoglobulin heavy chain gene locus (IgH). Specifically, during CSR, AID induces DNA breaks at the switch (S) regions within the Ig locus, enabling the rearrangement of antibody constant regions and subsequent alteration of antibody effector functions. However, while indispensable for immune function, AID's activity can also lead to genomic instability, as its actions may result in off-target DNA breaks and oncogenic rearrangements. Locus-specific conformation and repetitive S region-associated secondary structures, such as RNA: DNA hybrid/R-loop, are pivotal in promoting locus-specific DNA breaks, repair-recombination, and eventually Ig isotype switching. Therefore, understanding these dynamics is central to the regulatory mechanism that governs both CSR and lymphomagenic recombinations.

2.研究の目的

The purpose of the study is to investigate the regulation of structural dynamics at the IgH locus and its correlation with AID-induced DNA break and repair mechanisms, which are closely associated with CSR and oncogenic rearrangements leading to lymphomagenesis. R-loop formation, known to occur at the IgH locus, potentially facilitates AID-induced DNA breaks, contributing to genomic instability at both IgH and non-IgH loci. Recent research indicates that the R-loop structure may influence DNA repair processes necessary for joining AID-induced DNA double-strand breaks and CSR. Therefore, the study aims to elucidate the regulatory mechanisms governing Igh locus conformation, R-loop formation, and its potential impact on NHEJ-mediated repair, with the expectation of gaining novel insights into CSR and oncogenic rearrangements.

3.研究の方法

[3-1] The investigation into the function of HNRNPU involved the utilization of siRNA to induce its depletion, followed by the analysis of CSR, SHM, and cMyc/IgH translocation. The role of HNRNPU in regulating R-loop formation in the S regions was demonstrated through the performance of RNA/DNA hybrid IP or DRIP assays using established methods, and the results were verified through RNase H sensitivity. HNRNPU's role in NHEJ-mediated DNA repair was established by analyzing the CSR junctions and comparing them with the results obtained from 53BP1 and Shieldin complex deficient B cells. The interaction between HNRNPU and several NHEJ factors, including 53BP1 and the Shieldin complex, was assessed through standard co-immunoprecipitation. The recruitment of HNRNPU and NHEJ factors to the IgH locus was conducted via ChIP analysis. Finally, the assessment of RNA-dependent repair complex formation by HNRNPU and its susceptibility to LLPS-perturbing drugs was carried out, and the contribution of the RGG domain in HNRNPU was evaluated through the generation of appropriate mutants.

[3-2] Functional characterization of MED12 in CSR and AID-induced genomic instability was undertaken through siRNA-mediated knockdown and the examination of a series of reported mutants linked to cancer and XLID syndrome. The investigation into the importance of MED12 in AID-induced DNA breakage at the IgH locus and S-S synapsis was conducted through locus-specific DNA break assays and chromosomal conformation analysis, respectively. The recruitment of MED12 and its associated complex in the S region and at the 3’RR super enhancer was performed using

ChIP assays. The regulation of the 3’RR super-enhancer was monitored through the CRISPR/dCas9-p300 activator system and ASO-mediated eRNA depletion.

4.研究成果

[4-1] The RNA-binding protein HNRNPU promotes S-S joining mediated by C-NHEJ through the 53BP1-Shieldin DNA-repair complex. Importantly, the binding of HNRNPU to the RNA/DNA G-quadruplexes in the S region contributes to regulating the R-loop and accumulating single-stranded DNA at the locus. HNRNPU, characterized as an intrinsically disordered protein, interacts with both C-NHEJ and R-loop complexes in an RNA-dependent manner. Notably, the recruitment of HNRNPU and the C-NHEJ factors exhibits high sensitivity to inhibitors of liquid-liquid phase separation, indicating the potential formation of DNA-repair condensates. HNRNPU likely facilitates CSR by forming and stabilizing the C-NHEJ ribonucleoprotein complex, thereby preventing excessive accumulation of R-loops. Persistent DNA breaks and aberrant DNA repair may occur without such regulation, leading to genomic instability.

[4-2] The transcriptional co-activator MED12 functions in CSR independently of its known kinase module-dependent action in transcription. MED12 regulates the activation of the IgH super-enhancer through p300-Jmjd6/Carm1 coactivator complexes. Loss of Med12 leads to decreased H3K27 acetylation and eRNA transcription, resulting in impaired AID-induced DNA breaks, S-S synapse formation, and interaction with 3’RR super-enhancer. CRISPR-dCas9-mediated 3’RR-enhancer activation fully restored CSR defect and the epigenomic and transcriptional dysregulation imposed by MED12 depletion. The eRNAs derived from 3’RR play an important role; interestingly, mutations associated with XLID syndrome in MED12 have defective eRNA transcription and CSR. In conclusion, MED12 is essential to IgH super-enhancer activation, which is required for optimal AID-induced DNA breaks, Ig class switching, and genomic stability in B cells.

Quebec, Montreal, CANADA
INVITED TALK:
The Role of Mediator in Class Switch Recombination
Haque F, Honjo T, and Begum NA

〔図書〕(計 1 件)

Molecular Mechanism of Activation Induced Cytidine Deaminase.
Begum NA, Kobayashi M, Nagaoka H, and Honjo T.
Molecular Biology of B Cells [Chapter 13]; pp 257-333
3rd Edition 2024; ELSEVIER, Academic Press.
ISBN 978-0-323-95895-0

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Haque, F., Honjo, T. and Begum, N. A.	４．巻 8(47):eadd1466
２．論文標題 XLID syndrome gene Med12 promotes Ig isotype switching through chromatin modification and enhancer RNA regulation.	５．発行年 2022年
３．雑誌名 Science Advances	６．最初と最後の頁 1-18
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/sciadv.add1466	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Begum, N. A., Haque, F., Stanlie, A., Husain, A., Mondal, S., Nakata, M., Taniguchi, T., Taniguchi, H., and Honjo, T.	４．巻 40:e106393
２．論文標題 Phf5a regulates DNA repair in class switch recombination via p400 and histone H2A variant deposition	５．発行年 2021年
３．雑誌名 EMBO J	６．最初と最後の頁 1-21
掲載論文のDOI（デジタルオブジェクト識別子） 10.15252/embj.2020106393	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　3件）

１．発表者名 Farazul Haque, Tasuku Honjo, Nasim A. Begum
２．発表標題 The Role of Mediator in Class Switch Recombination
３．学会等名 Antibody Diversification and DNA deaminases in Immunity and Cancer（招待講演）（国際学会）
４．発表年 2022年

１．発表者名 Ahmed M. Refaat, Nasim A. Begum, Afzal Husain, Mikiyo Nakata, Hidetaka Kosako and Tasuku Honjo
２．発表標題 HNRNPU Facilitates Antibody Class Switch Recombination through C-NHEJ Promotion and R-loop suppression
３．学会等名 The 45th Annual Meeting of the Molecular Biology Society of Japan（国際学会）
４．発表年 2022年

１．発表者名 Ahmed M. Refaat, Nasim A. Begum, Afzal Husain, Mikiyo Nakata, Hidetaka Kosako and Tasuku Honjo
２．発表標題 HNRNPU Promotes Antibody Class Switch Recombination through C-NHEJ Promotion and R-loop suppression
３．学会等名 The 51st Annual Meeting of The Japanese Society for Immunology（国際学会）
４．発表年 2022年

〔図書〕　計1件

１．著者名 Begum NA, Kobayashi M, Nagaoka H, and Honjo T.	４．発行年 2024年
２．出版社 ELSEVIER, Academic Press.	５．総ページ数 77
３．書名 Molecular Biology of B Cells [Chapter 13]	

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関