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論文題目	HNRNPU Facilitates Antibody Class Switch Recombination through C-NHEJ Promotion and R-loop Suppression (HNRNPU 蛋白は、DNA 修復と R-loop 調節を介して CSR を促進する)		

(論文内容の要旨)

Activated mature B cells undergo various AID-induced genomic alterations, including base changes through point mutations (SHM) and recombination either in *cis* (CSR) or *trans* (*IgH/c-Myc*). Although AID-induced DNA lesions initiate both point mutation and recombination at the *IgH* locus, CSR requires additional steps, such as S-S synapsis and classical non-homologous end joining (C-NHEJ). Therefore, S-S joining by C-NHEJ-mediated DNA repair can tremendously influence the outcome of the CSR and associated chromosomal translocations. The RNAs at the DNA break have an impact on DNA repair either by recruiting RNA binding proteins (RBP) or by functioning as a template for DNA repair. Recently, AID's action was found to be regulated by a specific group of HNRNP family of RNA-binding AID co-factors. Heterogeneous nuclear ribonucleoprotein U (HNRNPU) is the largest member of HNRNP proteins, it can bind pre-mRNA and ssDNA, and plays a major role in nuclear matrix/scaffold attachment, telomere-length regulation, and RNA stability control. This study aimed to investigate the direct role of HNRNPU in CSR DNA repair and regulation of R-loop during antibody isotype switching. HNRNPU depleted B cells showed defects in C-NHEJ as revealed by the analysis of S-S junctions. Notably, the CSR junctions arise from shieldin subunit deficient B cells were relatively comparable in blunt joining and Microhomology usage with that in HNRNPU depleted cells. In addition, HNRNPU depleted B cells also showed an increase in S region single-strand DNA (ssDNA) and RPA accumulation, which phenocopies the deficiency of SHLD1 and SHLD2. Chromatin immunoprecipitation (ChIP) assay confirmed a marked elevation of HNRNPU occupancy at both Sμ and Sα upon CIT stimulation, which further confirmed a DNA damage response specific function of HNRNPU in CSR. HNRNPU interacts with C-NHEJ associated DNA repair factors, including LINP1 noncoding RNA. Notable HNRNPU interacting partners are KU80, DNA-PKCs, SHLD1, FEN1, DDX1, DHX9, SFPQ and NONO. Many of these interactions with HNRNPU were highly sensitive to RNase A treatment and HNRNPU without its RNA binding domain failed to interact with these partners, suggesting that HNRNPU's association with the C-NHEJ complex is RNA dependent. In line with this, deficiency of HNRNPU caused a significant reduction in NHEJ factors (53BP1 and KU80) occupancy at the recombining S regions. HNRNPU binds G4-RNA and G4-DNA in the switch GLT and the sense strand ssDNA, respectively. Loss of HNRNPU increases S region DNA:RNA hybrids (R-loops), contributing to the elevation of DNA end-resection during CSR. Moreover, the localization/occupancy of HNRNPU, 53BP1 and KU80 at the S region are sensitive to chemicals that disrupt liquid-liquid phase separation, suggesting the formation of DNA repair condensate at the S region during CSR

In summary, this study proposed two-step of HNRNPU functions in CSR. First, AID-induced DSBs increase HNRNPU occupancy at the S-regions, which serves as a noncoding RNA scaffold and stabilizes the C-NHEJ complex. Second, interacting with RNA and DNA G-quadruplexes promotes C-NHEJ-mediated DNA repair by preventing the expansion of S-region R-loops and excessive DNA end-resection. Most importantly, in the absence of HNRNPU, the NHEJ complex formation will be impaired, unprotected ends will go aberrant resection and would be incompatible with C-NHEJ, which would cause persistent DNA breaks and aberrant DNA repair, leading to genomic instability.

(論文審査の結果の要旨)

B 細胞がクラススイッチ組換え（CSR)によって機能的に異なる種類の抗体を産生するためには、ドナーおよびアクセプターのスイッチ領域 (S 領域) にて切断された DNA を再結合する、古典的な非相同末端結合（C-NHEJ）が必要である。

本研究により、RNA 結合蛋白質 HNRNPU が、53BP1-shieldin DNA 修復複合体を介した C-NHEJ により S-S 領域の結合を促進することが明らかになった。

HNRNPU は、その IDR ドメインにより、RNA 依存的に C-NHEJ および R ループ複合体の両方に相互作用する。HNRNPU は S 領域 RNA または DNA の G-４重鎖部分に結合することにより、R ループおよび ssDNA の蓄積を調節していた。

さらに HNRNPU と C-NHEJ に関わる因子の集積反応は、液ー液相分離阻害剤に非常に鋭敏であるため、DNA 修復濃縮体の形成が示唆される。このため、HNRNPU は C-NHEJ 因子と共に RNA タンパク質複合体を形成し安定化することにより CSR を促進し、過剰な R ループの蓄積を防ぐと考えられる。この R ループ調節により、持続的な DNA 切断や異常な DNA 修復、ひいては、ゲノムの不安定化が防がれているものと予想される。

以上の研究は、HNRNPU が CSR に果たす役割や DNA の修復と再結合に関する理解に大きく貢献し、今後の抗体遺伝子多様化の研究に大きな影響を与えることが期待される。

したがって、本論文は博士（医科学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 5 年 03 月 27 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。