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研究課題名（和文）ＡＩＤによるゲノム再構成に伴うクロマチン制御

研究課題名（英文）Transcription coupled chromatin regulation in AID induced genome rearrangement

研究代表者
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研究成果の概要（和文）：AIDによって誘導され転写されうるIgH領域のゲノム再編成はDNA切断と組換えの過程を経る。本研究の目的はAIDに誘導されるSHMとCSR転写と会合したクロマチン領域の同定である。DNA切断中のAID標的となる領域はSPT6,FACT,H3K4me3と3.3に富んでいる事を見いだした。SPT6,FACTはIgH領域のエピジェニックな厳密性を認識するヒストンシャペロンであるが、SPT6は特異的にAID領域が必須である。一方、AcH4リダーBRD4はUNG、IgH上のDNA修復蛋白のリクルートの促進が必要である。故に、DNA切断・修復に関わる標的領域はAIDによるゲノムの不安定性制御に必須である。

研究成果の概要（英文）：AID is the key enzyme required for antibody gene diversification by mutation (SHM) and recombination (CSR). AID induced SHM and CSR of transcriptionally active Ig heavy chain locus (IgH) occur via complex DNA cleavage and recombination. The study aimed to explore transcription-coupled chromatin features involved in AID induced genomic rearrangements. It revealed that the AID target loci that undergo DNA break are enriched with SPT6, FACT, H3K4me3 and H3.3. While SPT6 and FACT like histone chaperones are required for the epigenetic integrity of the AID target loci, SPT6 is specifically required for the AID locus for its expression. Moreover, H4 acetyl reader BRD4 promoted recombination phase of CSR by recruiting critical DNA repair proteins, UNG and 53BP1. Therefore, target loci associated histone epigenetic marks and specialized chromatin proteins are playing important role in AID induced genomic instability regulation.

研究分野：Immunology and Genomics

キーワード：AID　SHM　CSR　H3K4me3　FACT　SPT6　BRD4　UNG

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1.研究開始当初の背景

Gene rearrangement in immunoglobulin heavy chain locus is induced by AID in activated mature B cells, which is required for antibody memory formation (1,2). Although initiation of the genomic alteration occurs by targeted DNA break; off-target DNA breaks often lead to oncogene activation and lymphomagenic translocations (3,4). It revealed that AID induced DNA break is regulated by histone chaperone complexes associated with transcription elongation and the break-prone areas share a common chromatin signature with V(D)J and meiotic recombination hot spots (5-7).

2.研究の目的

Therefore, the purpose of the study is to identify critical chromatin modulators involved in AID induced antibody gene diversification process and to elucidate the chromatin regulatory mechanism that protects genome from aberrant genomic rearrangements.

3.研究の方法

The main focus of the proposed project was to explore the chromatin regulatory mechanism of the genomic loci subjected to AID induced DNA break and recombination. Functional group specific siRNA screening approach was applied to identify histone epigenetic reader proteins involved in CSR, SHM and IgH locus associated translocations. At first, CSR, SHM and translocation efficiencies were monitored for the promising candidate co-factors. Later, multiple DNA break assays were conducted in order to identify the DNA cleavage specific chromatin co-factors. Knockdown of chromatin proteins that blocked CSR without inhibiting the DNA cleavage of IgH locus were further analyzed for their ability to support nonhomologous end joining (NHEJ) or switch region synapsis by 3C assay. Chromatin regulation by histone chaperones or candidate chromatin proteins was verified by locus specific ChIP analysis along with the various histone-PTMs. Downstream mechanistic analysis strategy was designed based on ia candidate protein's placement, upstream or downstream of the AID induced DNA break.

4.研究成果

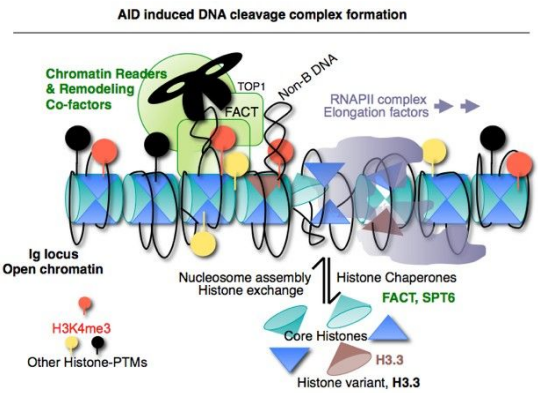
(1) For the first time, we showed that transciption elongation associated chromatin histone chaperones FACT and SPT6 (5,8), and elongation complex SPT4-SPT5 (6) are important for CSR and SHM, and they are critically involved in H3K4me3 regulation of the IgH locus. Loss of SPT6 also reduced the mutation frequency of many non-IgH AID targets, which correlated well with the H3K4me3 down regulation of the loci. Interestingly, SPT6 was found to be a unique histone chaperone capable of regulating the histone epigenetic state of both AID targets and the AID locus. Moreover, many SHM target zones were found to be co-enriched with FACT and histone variant H3.3 (9), indicating high histone turnover.

Critical co-factors: Specialized histone chaperones and elongation factors (5,6,8).

Critical chromatin feature : Active chromatin mark (H3K4me3) and rapid histone exchange (H3.3) (5,8,9).

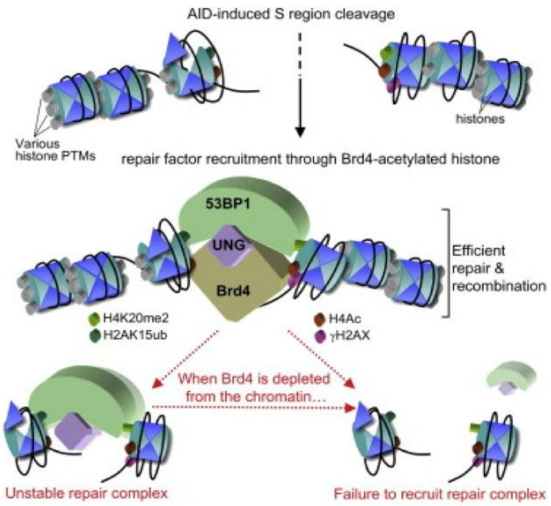
Critical DNA feature: Top1 regulated supercoil non-B DNA structure (10-11)

Taken together, the results demonstrate an important link between transcription coupled chromatin features of the target locus and AID induced DNA cleavage, which leads to genomic instability.



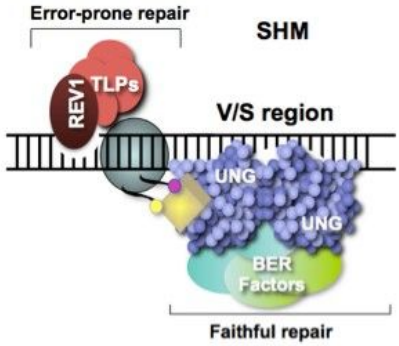
(2) However, the involvement of chromatin adaptors at the repair phase of AID induced genomic instability remains unknown. We identified acetylated histone reader BRD4 as a critical nonhomologous end-joining (NHEJ)

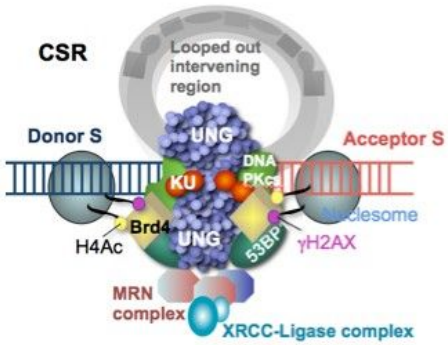
repair regulator of AID- and I-SceI-induced DNA breaks (12). BRD4 was recruited to the DNA break regions, and its depletion from the chromatin caused CSR impairment without affecting the DNA break generation. Inhibition of BRD4 suppressed the accumulation of 53BP1 and UNG at the switch regions, perturbed the joining between donor and acceptor switch (S) regions and also reduced aberrant translocations. *Therefore, BRD4 serves as a chromatin platform required for the recruitment of repair components during CSR and general DNA damage.*



The work also demonstrates that improper administration of BRD4 inhibitor JQ1, which is a popular therapeutic drug in various lymphoma/leukemia, may induce life threatening immunocompromised state due to the inhibition of all Ig-isotype production (12).

(3) AID induced aberrant translocations were completely blocked in the absence of Uracil DNA glycosylase (UNG), a repair enzyme that showed a non-enzymatic mode of action in AID induced CSR/SHM pathway (13-15). Further study reveled that a non-canonical scaffold function of UNG was responsible to regulate SHM negatively, but CSR positively (16). The SHM suppressive function of UNG is attributed to the recruitment of faithful base excision repair (BER) components at the cleaved DNA locus, which possibly outcompetes the error-prone polymerases. On the other hand, the CSR-promoting function of UNG enhanced recombination by promoting recruitment of the synapse factors like 53BP1 and DNA-PKCs. Similar to WT, several loss-of-catalysis mutants of UNG also showed hypermution suppressive and recombination promoting functions (16-17).





We propose that UNG (monomer/dimer) may remain in separate nuclear complexes in order to exert differential functions. UNG possibly forms distinct scaffold-complexes on the chromatin of IgH locus, which eventually regulates the steps after AID-induced DNA cleavage: suppression of error-prone repair in SHM and enhancement of end-joining recombination in CSR.

Conclusion & perspective:

It appears that the DNA break and repair phase specific chromatin proteins and histone epigenetic marks are playing an important role in AID induced genomic instability regulation during antibody gene diversification in B cells. However, there is little information regarding regulatory nature of chromatin in AID induced DNA break. Further study is necessary to fully understand the mode of positive and negative regulatory feature of the target chromatin that allows restricted genomic instability; while its deregulation leads to oncogenic mutations and aberrant genomic rearrangements.

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〔図書〕(計 2 件)

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〔その他〕

ホームページ等
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6.研究組織

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