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FACT and H3.3

New markers for the somatic hypermutation

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The antigen stimulation-induced genetic diversification of the immunoglobulin (Ig) genes establishes the antibody memory, which is critical to rapid and efficient immune response upon subsequent exposure to the antigen. Although extensive studies have been performed about the molecular mechanism of activation-induced cytidine deaminase (AID),[1] which is essential to establishment of antibody memory, the fundamental question as to how the AID-induced genetic diversity almost exclusively targets the Ig genes still remains to be fully answered. In our recent study, we found that transcription-coupled histone exchange is required for targeting the Ig genes[2] upon somatic hypermutation (SHM).

All 3 processes of AID-induced genetic diversity (i.e., SHM, class switch recombination [CSR] and gene conversion [GC]), is dependent on transcription of the target region, implying that certain transcription-related genetic or epigenetic hallmark(s) on the Ig genes serves as a target marker for the genetic diversity. Indeed, recent studies have identified markers that are required for SHM and CSR.

First, DNA sequences that are prone to form irregular DNA structure called non-B DNA during transcription is found in the Ig genes.[3] Formation of non-B DNA structure is likely to be required to initiate the DNA cleavage process mediated by DNA topoisomerase I.[4] Second, we found that the trimethylation modification at the Lys4 residue in histone H3 (H3K4me3) promotes SHM and CSR.[5] In Ig genes, H3K4me3 is especially enriched on the V(D)J and Sμ regions, which are the primary targets of the AID activity. Blocking H3K4me3 led to inhibition of CSR. Third, we and others reported that CSR and SHM occurs more frequently in the regions where the transcription elongation factor the FACT complex and Spt5 accumulate.[2,5,6] Since these epigenetic marks are commonly found in transcriptionally active regions, it was anticipated that there would be additional markers that are more specific to the Ig genes.

Facilitates chromatin transcription (FACT) is a heterodimeric protein complex composed of SSRP1 and Spt16 and was originally identified from its biochemical activity to help RNAPII transcription to elongate on a chromatin template. It is proposed that FACT disrupts the nucleosome in front of the elongating RNAPII and reverts it after progression of the polymerase. We previously identified that FACT is required for CSR.[5] FACT knockdown dramatically reduced CSR efficiency as well as the histone modification H3K4me3, which was subsequently found to be required for CSR.

We recently found that FACT also promotes SHM and accumulates specifically on the SHM target regions on the Ig genes.[2] From candidate-based screening for the SHM-promoting transcription elongation factors, we found that depletion of FACT most strongly reduced the mutation on the SHM reporter transgene in human BL2 lymphoma cells. Importantly, we observed strong FACT occupancy on the immunoglobulin heavy chain (*Igh*) loci between the V(D)J region and Eμ intronic enhancer, and the 5′ side of the Sμ switch region, both of which accumulate SHM upon AID expression. Meanwhile, FACT occupancy was only weak at highly transcribed non-Ig genes that bear high RNAPII, Spt5, and H3K4me3 occupancies, showing that the FACT accumulation on *Igh* is a gene-specific event rather than a general feature of transcription. Accumulation of FACT suggested that the nucleosomes in the SHM target region is more rapidly exchanged than those in other regions. Indeed, accumulation of the histone variant H3.3, a hallmark of replication-independent histone turnover, coincided with the FACT enrichment. This dual enrichment of FACT and H3.3 was observed not only in *Igh,* but also the kappa light chain locus in mouse spleen B cells.

Our previous genome-wide analysis revealed that there are at least 4 non-Ig SHM targets in the BL2 genome that can be mutated as efficiently as the V(D)J region by the C-terminally truncated AID mutant transgene carrying enhanced SHM-inducing activity.[7] We could not observe enriched FACT and H3.3 occupancies on them, and found that these non-Ig loci are not efficiently mutated by the wild-type AID transgene, which can mutate *Igh*.[2]

Taken together, these data suggest that rapid histone exchange makes the genomic region sensitive targets to SHM. Then, why does SHM prefer nucleosome-unstable regions on the Ig genes? We speculate that the nucleosomal DNA is protected from SHM. Moreover, unwinding DNA from a nucleosome produces one negative supercoil, which can eventually produce non-B DNA that promotes

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SHM. Whether the artificial enhancement of histone exchange would increase SHM remains to be tested.

With this finding of the new SHM marker, we can now propose a more detailed mechanism for SHM targeting: SHM is generated by error-prone repair after Top1 cleavage at non-B DNA induced by excessive transcription and Top1 reduction by AID, with the support of certain H3K4me3-binding factors. The Ig genes are the primary SHM targets, because their DNA is more frequently exposed from nucleosome and thus contains more negative supercoil. It would be interesting to test whether FACT and H3.3 is involved in the V(D)J recombination as well. It is well known that the V(D)J recombinase RAG binds to H3K4me3. Moreover, a recent study suggests that transcription-coupled disruption of nucleosome allows RAG to attack the recombination signal sequence.[8] Future study will elucidate the molecular mechanism of how FACT and H3.3 accumulate on the Ig genes. Collectively, our finding shed light on the importance of histone exchange dynamics in the SHM-targeting mechanism.

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