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Cutting Edge: CTNNBL1 Is Dispensable for Ig Class Switch Recombination

Li Han, Shahnaz Masani, and Kefei Yu

Ig class switch recombination (CSR) and somatic hypermutation require activation-induced cytidine deaminase (AID). The search for AID-interaction factors has been a major research effort in the field, as the mechanism of preferential targeting of AID to Ig loci remains elusive. CTNNBL1 is one of the few identified AID-interacting factors and has been shown to affect AID-mediated mutation and gene conversion in chicken DT40 cells. CTNNBL1 was also implicated in mammalian CSR by the fact that an AID mutant that fails to interact with CTNNBL1 also fails to support CSR in AID-deficient mouse B cells. To directly assess the role of CTNNBL1 in CSR, we disrupted the CTNNBL1 gene on both alleles in mouse CH12F3 cells by gene targeting. We found normal levels of CSR in CTNNBL1-deficient cells, indicating that CTNNBL1 is dispensable for CSR. The Journal of Immunology, 2010, 185: 1379–1381.

An Ag-stimulated mature B cell can further diversify its Ig gene by class switch recombination (CSR), somatic hypermutation (SHM), or gene conversion (GC). CSR is a cut-and-paste chromosomal deletion event that allows a B cell to use an alternative C region (γ, e, α) located downstream of the default µ C region, thereby changing the expressed Ig isotype from IgM to IgG, IgA, or IgE (1). SHM introduces mutations in Ig variable regions to allow improved affinity for Ag binding. In birds, Ig diversification occurs predominantly through templated gene conversion (2).

All three processes (CSR, SHM, and GC) require local transcription and a lymphoid-specific factor called activation-induced cytidine deaminase (AID) (3, 4). AID was identified in a subtractive cDNA library screening as an early upregulated gene when a mouse B cell line (CH12F3) was induced to undergo CSR (5). Cumulative genetic and biochemical findings indicate that AID is a cytidine deaminase that converts cytidines to uracils in DNA at specific regions (6–8). CSR, SHM, and GC are all tightly associated with transcription. Purified AID deaminites cytidines only on single-stranded DNA (9, 10), suggesting that the need for transcription is likely to temporarily separate the two DNA strands. The kilobase-long switch regions that are the main targets for CSR contain many GC-rich repetitive sequences. They tend to form stable secondary structure, such as R-loop, upon transcription (11, 12). The R-loop structure could provide stable extensive single-stranded DNA region as optimal substrate for AID, which may partly explain the targeting mechanism of AID in CSR (1). However, it is more difficult to explain how AID targets Ig variable regions, which do not form R-loop structures. Therefore, what distinguishes Ig loci as preferred AID targets versus other highly transcribed regions in the genome remains an enigma.

It is well known that mutations in different regions of AID differentially affect SHM or CSR, which prompted a hypothesis that AID is differentially recruited in SHM or CSR by different accessory factors (13–15). Recently, an AID-interacting factor called Cnmb1 was identified through yeast two-hybrid screening (16). Very little is known about the cellular function of Cnmb1, which could involve apoptosis and obesity according to several reports (17, 18). Of the few AID-interacting factors reported in the literature, Cnmb1 is of particular interest because of the direct genetic evidence that Cnmb1-deficient chicken DT40 cells have a markedly reduced rate of AID-mediated mutation and gene conversion (16). Additionally, an AID mutant harboring amino acid substitutions at residues 39–42 (AID39/42) that fails to interact with Cnmb1 also fails to rescue CSR in AID-deficient mouse primary B cells (16). There was also evidence that Cnmb1 is a component of a spliceosome complex (16, 19). This is particularly interesting because there has been a 15-yr-old mystery as to why CSR requires RNA splicing of the noncoding switch region transcripts known as germline transcripts (20–22).

To determine whether Cnmb1 is required for CSR, we knocked out both copies of Cnmb1 gene in mouse CH12F3 cells by somatic gene targeting. We found that Cnmb1-deficient CH12F3 cells are normal, if not slightly more efficient in CSR to IgA, thus providing direct evidence that Cnmb1 is dispensable for CSR.

Materials and Methods

Cell culture and CSR assay

The CH12F3 cell line was a gift from Dr. T. Honjo (Kyoto University, Kyoto, Japan). Cell culture conditions, CSR, and cell proliferation assays have been described previously (23).

Abbreviations used in this paper: AID, activation-induced cytidine deaminase; CSR, class switch recombination; DTA, diphtheria toxin; GC, gene conversion; Puro, puromycin resistance gene; SHM, somatic hypermutation.

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Gene targeting

A 5.8-kb and a 1.8-kb DNA fragment were PCR amplified from CH12F3 genomic DNA and cloned into a targeting vector as homology blocks for gene targeting (Fig. 1A). Procedures of two rounds of gene targeting to knock out a gene in CH12F3 cells has been described in detail in a previous study (23).

RT-PCR

Total cellular RNA was extracted using TRizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. One microgram of total RNA was reverse-transcribed into cDNA with random hexamers and SuperScript II reverse transcriptase in a 20-μl reaction (Invitrogen). Two microliters of the

FIGURE 1. Gene targeting of Ctnnbl1 in CH12F3 cells. A, Genomic organization of wild-type and targeted mouse Ctnnbl1 locus. Small triangles indicate loxP sites. Restriction enzyme sites are indicated by B for BamHI and H for HindIII (shown are only the relevant ones). DTA, diphtheria toxin; Puro, puromycin resistance gene. B, Southern blot analysis. Left panel, HindIII-digested genomic DNA hybridized with the 5′ probe. Right panel, BamHI-digested genomic DNA hybridized with the 3′ probe. Genotype symbols: +, wild-type allele; P, targeted allele with puromycin selection cassette; Δ, targeted allele with puromycin selection cassette removed. C, RT-PCR. CTNNBL1 coding region and part of β-actin (as loading control) were amplified from random-primed cDNA with 30 cycles of PCR.

FIGURE 2. Normal proliferation and CSR in Ctnnbl1-deficient cells. A, Cell proliferation. Cells were seeded at 5 × 10⁴/ml and live cell numbers were counted every 24 h over 3 d. Error bars represent standard deviations from three independent experiments. B, Class switch assay. Representative FACS analysis of CSR by cell surface staining of IgA after 72 h of cell growth with or without cytokines. Numbers in boxed areas indicate percentages of IgA-positive cells. CIT, anti-CD40 Ab, IL-4, and TGF-β1.
Results and Discussion

Gene targeting of Ctnnbl1 gene in CH12F3 cells

Mouse Ctnnbl1 gene contains 16 exons spanning a region of ~150 kb on chromosome 2 (Fig. 1A). Little is known about the cellular function of Ctnnbl1 or what residues might be of any functional importance. We chose to delete exons 8–10 and part of exon 7 of the Ctnnbl1 gene (Fig. 1A) based on the convenience of finding homology blocks that are scarce in repetitive sequences. The targeted deletion should abolish at least 60% of the coding capacity of the Ctnnbl1 gene and result in a null allele. Two rounds of gene targeting were performed using a strategy that has been described previously (23). Briefly, after targeting of the first allele (+/P), a Cre-expressing plasmid was transiently transfected to remove the puromycin selection cassette flanked by a pair of loxP sites. The resulting puromycin-sensitive clone (+/Δ) was retargeted by the same targeting vector to obtain a double knockout clone (P/Δ). Cell genotypes were first screened by PCR and then confirmed by Southern blot analysis (Fig. 1B). The assays described in this study were carried out using a Ctnnbl1-haplodeficient clone (+/P) and a double knockout clone (P/Δ), which are siblings in the second round of gene targeting. Cells that are targeted on both alleles (P/Δ) lack mature Ctnnbl1 transcript based on PCR amplification of the reverse-transcribed cDNA (Fig. 1C), indicating that the targeted deletion resulted in a null allele.

Ctnnbl1-deficient cells proliferate normally (Fig. 2A) based on microscopic counting of live cells every 24 h over a span of 3 d. When cells are induced to undergo CSR with the addition of anti-CD40 Ab, IL-4, and TGF-β1, Ctnnbl1-deficient cells showed normal, if not slightly higher, efficiency in CSR (Fig. 2B). Identical results were obtained with Ctnnbl1 Δ/Δ cells, which have the puromycin selection cassette removed by the Cre-LoxP reaction (not depicted). These data provide direct evidence that Ctnnbl1 is dispensable for CSR.

Direct testing of Ctnnbl1 in CSR has been difficult in mouse models because Ctnnbl1 is required for mouse embryo development (A. Chandra, C. Rada, and M. Neuberger, personal communication). In the earlier study that identified Ctnnbl1–AID interaction (16), Ctnnbl1 was not only found to affect the rate of AID-mediated SHM and gene conversion. Mol. Cell. 12: 501–508.

However, this apparent discrepancy could be explained by a number of possibilities. First, Ctnnbl1 might be specially involved in the SHM/GC process but not in CSR. This would explain diminished SHM/GC in Ctnnbl1-deficient DT40 cells, but normal CSR in Ctnnbl1-deficient CH12F3 cells. Second, the CSR defect associated with AID39/42 mutation might be attributable to an intrinsic defect in that mutant AID, rather than to its interaction with Ctnnbl1. Finally, we could not rule out the possibility that there is a functionally redundant gene (or genes) in mice that could compensate for the loss of Ctnnbl1. Of course in such case, the AID39/42 mutant would have to lose the ability to interact with all of these CTNNBL1-like factors. Resolution of these possibilities awaits further investigations.

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Disclosures

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