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Biased dA/dT somatic hypermutation as regulated by the heavy chain intronic iEμ enhancer and 3′Eα enhancers in human lymphoblastoid B cells

Atsumasa Komori [1,2], Zhenming Xu [1], Xiaoping Wu, Hong Zan, Paolo Casali *

Center for Immunology, School of Medicine and School of Biological Sciences, University of California, 3028 Hewitt Hall, Irvine, CA 92697-4120, USA

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Abstract

Somatic hypermutation (SHM) in immunoglobulin gene (Ig) variable (V) regions is critical for the maturation of the antibody response. It is dependent on the expression of activation-induced cytidine deaminase (AID) and translesion DNA polymerases in germinal center B cells as well as Ig V transcription, as regulated by the Ig heavy chain (H) intronic enhancer (iEμ) and the 3′ enhancer (3′Eα) region. We analyzed the role of these *cis* elements in SHM by stably transfecting Ramos human lymphoblastoid B cells with a rearranged human IgH chain VD (diversity) J (joining) DNA construct containing a V_H promoter at the 5′ end and C_H1 and C_H2 exons of Cγ1 at the 3′ end. In this construct, mutations preferentially targeted dA/dT basepairs in the RGYW/WRCY hotspot. Most of the dA/dT mutations and accompanying dC/dG mutations were transitions. Deletion of iEμ resulted in decreased SHM which could be partially restored by insertion of the IgH hs1,2 enhancer. Other two 3′Eα enhancers, hs3-hs4, did not significantly increase the mutation frequency, but further strengthened the dA/dT bias. The frequency and spectrum of the mutations were independent of the genomic integration of the transgene or V gene transcription level. Thus, we have established a novel in vitro system to analyze SHM and identify the role of multiple *cis*-regulatory elements in regulating dA/dT biased SHM. This model system will be useful to further address the role of other *cis*-regulating elements and recruited *trans-acting* factors in expressing the modalities of SHM.

Keywords: 3′ Heavy chain enhancer; *cis*-Regulatory elements; iEμ; Intronic Ig μ enhancer; SHM; Somatic hypermutation

1. Introduction

During B cell development in the bone marrow, recombination-activating genes 1 (RAG1) and RAG2-mediated DNA recombination of germline immunoglobulin V, D and J genes underlies the generation of the high degree of diversity (in excess of 10^9) of the B cells receptor (BCR) for antigen and the primary antibody repertoire. The impact of antigen in the specialized microenvironment of the germinal center leads to diversification of such a primary BCR repertoire and the generation of antibodies with higher affinities through somatic hypermutation (SHM) and different immunological effector functions through class switch DNA recombination (CSR). SHM targets the V(D)J sequence immediately downstream of the Ig V promoter and extends to the intronic DNA downstream the J_H, $J_κ$ and $J_λ$ region. It inserts mutations at a rate of 10^{-4} to 10^{-3} change/base/cell division and displays a striking preference for the RGYW/WRCY mutational hotspot (Rada et al., 1994; Rogerson, 1994; Wagner et al., 1995; Foster et al., 1999; Diaz and Casali, 2002). CSR substitutes the IgH Cμ region with downstream Cγ, Cα and Cε regions, thereby generating antibodies with different effector functions.

SHM and CSR are dependent on activation-induced cytidine deaminase (AID) (Muramatsu et al., 2000; Revy et al., 2000), which is strictly expressed in differentiating germinal

Abbreviations: AID, activation-induced cytidine deaminase; BCR, B cell receptor; BER, base excision repair; DSB, double strand break; iEμ, intronic Ig μ enhancer; Ig, immunoglobulin; MMR, mismatch repair; SHM, somatic hypermutation; SSCP, single-strand conformational polymorphism

* Corresponding author. Tel.: +1 949 824 4456; fax: +1 949 824 2305.
E-mail address: pcasali@uci.edu (P. Casali).
[1] These authors contributed equally to this work.
[2] Present address: Department of Advanced Medical Research, Clinical Research Center, National Nagasaki Medical Center, Japan.

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center B cells (Muramatsu et al., 1999). AID is a sequelog (Varshavsky, 2004) of the RNA-editing cytidine deaminase APOBEC1 (Muramatsu et al., 1999). As such, AID was thought to edit an unknown mRNA precursor to yield novel mRNAs, which would in turn encode endonucleases to cleave targeted DNA at hypermutating V(D)J regions or switch (S) regions (RNA editing hypothesis) (Honjo et al., 2002, 2004; Doi et al., 2003; Begum et al., 2004; Nagaoka et al., 2005). However, mounting evidence indicates that AID directly deaminates DNA dC residues (Petersen-Mahrt et al., 2002; Pham et al., 2003), yielding dU:dG mispairs, which are replicated over or dealt with either the base excision repair (BER) or the mismatch repair (MMR) pathways to introduce mutations through the intervention of translesion DNA polymerases, such as pol ζ, pol η and pol θ (Rada et al., 2004). In addition, accumulating evidence suggests that mutations can be introduced by the same translesion DNA polymerases (Zan et al., 2001; Diaz and Casali, 2002; Diaz and Lawrence, 2005) while repairing DNA breaks, including double stranded DNA breaks (DSBs) involving resected ends generated through AID-dependent DNA deamination (Bross et al., 2000; Papavasiliou and Schatz, 2000; Wu et al., 2003; Zan et al., 2003; Nagaoka et al., 2005; Xu et al., 2005).

SHM depends on V gene transcription (Peters and Storb, 1996; Fukita et al., 1998), as suggested by the greatly diminished frequency of mutations in the IgH locus when the V gene promoter is removed (Fukita et al., 1998), and conversely, by unchanged level of SHM in V regions if the endogenous promoter is replaced with a transcriptionally active heterologous promoter (Betz et al., 1994; Tumas-Brundage and Manser, 1997). B cell specific V_H gene transcription is regulated by the IgH intronic enhancer (iEμ), which is located 5′ of Sμ (Banerji et al., 1983; Gillies et al., 1983) and recruits multiple transcription factors, including proteins of the E-box and POU families (Ernst and Smale, 1995). A second IgH transcription regulatory region is located 2 kb downstream of the Cα gene in the mouse and consists of four B cell-specific DNase I hypersensitivity (hs) sites, hs3a, hs1,2, hs3b, and hs4, each of which is referred to as an IgH 3′Eα enhancer. The hs1,2 enhancer lies at the center of a 25 kb dyad symmetric region with two virtually identical hs3a and hs3b located at the two termini (Chauveau and Cogne, 1996; Saleque et al., 1997). In the human, a duplicated 3′Eα region separates two C_H gene clusters, resulting in a germline C_H arrangement of Cμ-Cδ–Cγ3-Cγ1-Cα1–3′Eα-Cγ2-Cγ4-Cε–Cα2-3′Eα. Each 3′Eα region consists of three hs sites in the 5′-hs3-hs1,2-hs4-3′ configuration, with two hs1,2 enhancers being inverted in respect to each other (Chen and Birshtein, 1997; Mills et al., 1997). Different transcription factors, such as E2A, Oct, Ets, and NF-κB family proteins, regulate the IgH transcription through their binding motifs in the 3′Eα region at different B-cell differentiation stages (Khamlichi et al., 2000; Linderson et al., 2001; Schaffer et al., 2003; Kim et al., 2004; Sepulveda et al., 2004).

The iEμ has been implicated in SHM by the findings that it could replace the 3′ λ enhancer to induce SHM of a λ transgene in mice (Klotz and Storb, 1996), and by the finding that iEμ increased SHM in a human pre-B lymphoma cell line, 18–81, transfected with a rearranged Igμ gene under the regulation of a thimidine kinase (tk) promoter and a 3′ κ chain enhancer (Bachl et al., 1998). In mice, the addition of hs1,2 to transgenes, even to those containing iEμ, did not increase mutation frequency to level comparable to that of endogenous IgH (Tumas-Brundage et al., 1997; Terauchi et al., 2001). Further addition of hs3b-hs4 increased the mutation load by eight-folds, suggesting a role of hs3b-hs4 in SHM (Terauchi et al., 2001). However, in knockout mice lacking the hs3b-hs4 region, the mutation efficiency in the J_H intronic region is similar in the wildtype and targeted allele, suggesting that the function of hs3b-hs4 can be compensated by other IgH locus *cis*-elements (Morvan et al., 2003).

These preliminary data from different models prompted us to undertake a systematic analysis of the contribution of the IgH *cis*-elements in SHM. We reasoned that a human IgH construct that can be transfected into Ramos B cells, a spontaneously hypermutating human Burkitt's lymphoma cell line (Sale and Neuberger, 1998), would be a good vehicle to analyze the function of the iEμ, hs1,2 and hs3-hs4 enhancers in different combinations. We further reasoned that regulation of transcription of such IgH constructs by its own promoter would have higher physiological relevance. To this end, we cloned the V_HDJ_H DNA from a hybridoma that secrets a human IgG mAb against rabies virus, mAb57 (Ueki et al., 1990; Ikematsu et al., 1993) in a "physiological" configuration, that is, flanked 5′ by its own V_H promoter and 3′ by the iEμ enhancer, followed by a recombined Sμ-Sγ1 region preceding the first two exons of the Cγ1 cluster. This was immediately followed downstream by the 3′Eα hs1,2 or hs3-hs4 enhancers. Different iterations of this construct, containing or not containing the iEμ and/or 3′ enhancers, were used to stably transfect into Ramos B cells. This Burkitt's lymphoma cell line displays a germinal center-like phenotype and spontaneously hypermutates the rearrange and expressed Ig V(D)J genes in vitro. The frequency and the nature of V_H region mutations in stable Ramos B cell transfectants were analyzed to address the role of the iEμ and 3′Eα enhancers in SHM.

2. Materials and methods

2.1. Plasmid construction

Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen Inc.). The 5′-flanking V_H1 promoter, rearranged V_H1-DXP′1-J_H5, iEμ and first two exons of switched Cγ1 sequence DNA (P-VDJ-iEμ-Cγ1) were amplified *en block* by Elongase (Invitrogen Corp.) from the genomic DNA of the mAb57-secreting hybridoma, using the primer A (forward primer with *Kpn*I site: 5′-AGAGCTGGGTACCGCAGGATTTAGGGCTTGGTCTC-3′) and primer B (reverse primer with *Eco*RI site: 5′-AGAGCTGAATTCTTGGAGATGGTTTTCTCGATG-3′). A modified pcDNA3.1 vector (Invitrogen Corp., Carlsbad, CA) with the 667 bp *Mlu*I-*Nhe*I fragment of CMV promoter–enhancer excised, was used to clone and express P-VDJ-iEμ-Cγ1. To construct P-VDJ-Cγ1 which lacks iEμ,