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Activation induced cytidine deaminase mutant (AID-His130Pro) from Hyper IgM 2 patient retained mutagenic activity on SHM artificial substrate

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ABSTRACT

Activation induced cytidine deaminase (AID) is an essential enzyme for class switch recombination (CSR) and somatic hypermutation (SHM) during secondary immune response. Mutations in the *AICDA* gene are responsible for Hyper IgM 2 syndrome where both CSR and SHM or only CSR are affected. Indeed, triggering either of the two mechanisms requires the DNA deamination activity of AID. Besides, different domains of AID may be differentially involved in CSR and SHM through their interaction with specific cofactors. Herein, we studied the AID-induced SHM activity of the AID-His130Pro mutant identified in a patient with Hyper IgM 2 syndrome. AID mutagenic activity was monitored by the reversion of nonsense mutations of the EGFP gene assessed by flow cytometry. We found that the His130Pro mutation, which affects CSR, preserves AID mutagenic activity. Indeed, the His130 residue is located in a putative specific CSR region in the APOBEC-like domain, known to involve CSR specific cofactors that probably play a major role in AID physiological activities.

1. Introduction

Class Switch Recombination (CSR) and Somatic Hypermutation (SHM) are the mechanisms that allow antibodies to ensure diverse effector functions and to reach affinity maturation. CSR occurs by intrachromosomal deletional recombination within Switch (S) regions located upstream of constant heavy chain of immunoglobulins (Ig) genes (except Cδ) and leads to production of Ig isotypes other than IgM (Xu et al., 2012). SHM results mainly from single nucleotide substitutions within the variable (V) genes of immunoglobulins (Peled et al., 2008). These two mechanisms are initiated by the enzyme Activation Induced Cytidine Deaminase (AID) following interaction between CD40 and CD40L expressed on B cells and activated T cells respectively (Crouch et al., 2007; Muramatsu et al., 2000). Indeed, genetic defects of AID underlie Hyper IgM 2 (HIGM2) syndrome (Durandy et al., 2013; Muramatsu et al., 2000; Revy et al., 2000). AID is a protein of 24 kD encoded by *AICDA* gene and specifically expressed in activated B cells present within germinal centers of secondary lymphoid organs (Muto et al., 2000). Because of its mutagenic activity, AID expression and activity are tightly regulated at transcriptional and post-translational levels (Crouch et al., 2007; Zan and Casali, 2013). The cellular localization of AID is regulated by nuclear export signal (NES) and

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nuclear localization signals (NLS) which control the shuttling of the protein between the cytoplasm and the nucleus in an active manner (Ito et al., 2004; Patenaude et al., 2009). The catalytic domain of AID is common to the cytidine deaminase family. It includes a motif (His-X-Glu-..-Pro-Cys-X-X-Cys) involved in zinc coordination where His56, Cys87 and Cys90 bind Zn2+ and the carboxylic acid group of Glu58 serves as a general acid-base catalyst involved in DNA deamination (Wedekind et al., 2003). AID also shares an APOBEC-like domain with the cytidine deaminase family. This domain contain the hotspot recognition loop which contributes to the differential recognition of specific DNA sequence motifs (Kohli et al., 2009). How AID can target CSR and SHM in different ways put out the hypothesis of the presence of specific cofactors. Indeed, AID has been proposed to be a docking protein acting by the recruitment of specific cofactors which mediate either mechanism through interaction with different AID domains (Durandy et al., 2006; Shinkura et al., 2004). Analyses of AID mutant proteins activity in HIGM2 patients allow a better understanding of CSR and SHM targeting mechanism. Today, 45 mutations of the *AICDA* gene are reported (Caratão et al., 2013; Mahdaviani et al., 2012; Ouadani et al., 2015; Patiroglu et al., 2015; Trotta et al., 2016) and are mainly homozygous or compound heterozygous with an autosomal recessive inheritance, except two heterozygous mutations with a dominant negative effect (Imai et al., 2005; Kracker et al., 2010). Most of them lead to the loss of both CSR and SHM activities. Nevertheless, AID C-terminal mutations display a defect in CSR with normal SHM activity showing that this domain is CSR specific (Durandy et al., 2006). Indeed, the AID C-terminal domain is required for S region recombination (Sabouri et al., 2014). Interestingly, AID Arg112Cys mutation located outside the AID C-terminal domain seems to retain residual SHM activity (Mahdaviani et al., 2012; Mu et al., 2012). Otherwise, analysis of AID mutants generated by site-directed show that some AID N-terminal mutations retain CSR activity but lose SHM (Shinkura et al., 2004). Recently, we have identified a novel AID mutation (His130Pro), in HIGM 2 patients, located in the APOBEC-like domain (Ouadani et al., 2015). Fascinatingly, the His130 residue belongs to a putative RNA-binding region that would likely be CSR specific, through the interaction of AID with specific RNA (Zheng et al., 2015). We aim herein to assess the SHM activity of AID-His130Pro mutant using an in vitro mutagenesis assay on an artificial SHM substrate already developed by Rougeon and collaborators (Jovanic et al., 2008).

2. Materials and methods

2.1. Ethical note

Written consent was obtained before studies and adherence to the Declaration of Helsinki Principles was maintained.

2.2. AID protein analysis

EBV-lymphoblastoide cell lines (LCLs) were lysed for 5 min in 12.5 mM Tris HCl, 4% Glycerol and 0.4% SDS. Proteins were quantified using Bicinchoninic Acid Protein Assay Kit (Sigma), subjected to 15% SDS PAGE polyacrylamid gel and transferred to a PolyVinyliDene Fluoride (PVDF) membrane. AID and β-actin were revealed using respectively a rabbit monoclonal anti AID (Cell Signaling) and a rabbit monoclonal anti actin (Santa Cruz Biotechnology). Western blots were analyzed by enhanced chemiluminescence with Pierce ECL Western blotting reagent (Thermo Scientific).

The functional impact of the His130Pro mutation was predicted with the PolyPhen-2 (Adzhubei et al., 2010) and Sorts Intolerant From Tolerant Substitutions (SIFT) (Kumar et al., 2009) algorithms. These methods consider protein structure and/or sequence conservation information for each gene.

2.3. Plasmid constructs and site-Directed mutagenesis

Wild type (AID-wt) and mutant (AID-His130Pro) *AICDA* cDNA were amplified from EBV-LCLs using the following specific primers: Forward (5′GGGTTTGCTAGCATGGACAGCCTCTT3′); Reverse (5′ TACCCTCTCGAGCTATCAAAGTCCCA3′). PCR products were immediately cloned using TA cloning Kit (Invitrogen) following the manufacturer's specifications. The recombinant plasmids were purified from *Escherichia coli* XL1 using Wisard Mini prep Kit (Promega) and then sequenced using vector specific primers (M13 Forward 5′-GTAAAACGACGGCCAG-3′, M13 Reverse 5′-CAGGAAACAGCTATGAC-3′). DNA fragments were liberated from PCRII Topo vector using XhoI and NheI (Roche) restriction enzymes then ligated into XhoI-NheI digested pCINeo vector using T4 ligase (Roche). The recombinant plasmids were purified from *Escherichia coli* XL1 competent cells using Midi prep kit (Qiagen).

The recombinant pCINeo-AID-His56Tyr, in which the mutated AID gene contains the *c.166T > C* mutation, was generated from the recombinant pCINeo-AID-wt plasmid with the QuikChange site directed mutagenesis system (Stratagene, La Jolla, CA), according to the manufacturer's instructions, using the following primers: Forward (5′ GCAATAAGAACGGTGCTACGTGGAATTGCTCTTC3′) and Reverse (5′GAAGAGCAATTCCACGTAGCAGCCGTTCTTATTGC3′). This mutant was used as a negative control since the His56Tyr mutation alters both CSR and SHM (Durandy et al., 2006).

2.4. Jurkat stable cell lines establishment

Jurkat cells were cultured in RPMI glutamax with 10% FBS, 100 U/ml penicillin and 100 g/ml streptomycin at 37 °C and 5% CO_2. Stable Jurkat cell lines, expressing AID-wt (Jurkat AID-wt), AID-His130Pro (Jurkat AID-His130Pro) and AID-His56Tyr (Jurkat AID-His56Tyr), were obtained by nucleofection of 10^6 Jurkat cells with 2 μg of the corresponding recombinant pCINeo plasmids according to the manufacturer's instructions (Amaxa™ 4D-Nucleofector™ Protocol for Jurkat clone E6.1 from Lonza). Forty eight hours after transfection, selection was made by addition of Neomycin (Sigma-Aldrich) at a final concentration of 2 mg/ml. Medium was changed every 2–3 days until all cells of the negative control (non-transfected Jurkat cells) were dead. Cells were then amplified by batch culture in RPMI glutamax with 10% FBS, 100 U/ml penicillin, 100 g/ml streptomycin and 2 mg/ml Neomycin, at 37 °C and 5% CO_2. They were tested for *AICDA* gene integration and expression by reverse transcriptase (RT)-PCR and sequencing. AID proteins expression was assessed by western blot as described previously.

2.5. AID-induced mutagenesis analysis

SHM vectors used in this assay are the wild type vector pEM7-EGFP, which express the EGFP protein, and the mutant pEM7-TAG182 vector which is unable to express the protein (Jovanic et al., 2008).

2×10^6 of each Jurkat stable cell line were transfected with either 3 μg of pEM7-EGFP or pEM7-TAG182 by nucleofection as described above. Twenty four hours after transfection cells were centrifuged, washed by PBS, re-suspended in PBS with 0.5% SVF and analyzed by flow cytometry.