Karolinska Institutet

Department of Laboratory Medicine

Role of DNA repair in class switch recombination and somatic hypermutation

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid karolinska Institutet offentligen försvaras i Hörsal 4Z Solen, Alfred Nobels Allé 8, Karolinska Institutet Huddinge

Fredagen den 2 Dec 2011, kl 09:30

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Stockholm 2011

ABSTRACT

Class switch recombination (CSR) and somatic hypermutation (SHM), occurring in the germinal center, are two important processes for B cell development. Both are initiated by activation-induced cytidine deaminase (AID), through deamination of the C residues in the variable and switch regions of the immunoglobulin locus, resulting in either in single stranded or double stranded DNA breaks. At least three pathways (nonhomologous end joining (NHEJ), base excision repair (BER), and mismatch repair (MMR)) have been implicated in processing, repair and ligation of the DNA breaks during CSR and SHM. However, the way in which these pathways are regulated and coordinated to mediate CSR and /or SHM is still not well understood.

To explore the potential role of several proteins in CSR and SHM, including Ataxia-telangiectasia and Rad3-related (ATR), Artemis, Cernunnos and the Mre11-Rad50-NBS1 (MRN) complex, CSR junctions and SHM patterns in the immunoglobulin variable region gene were analyzed in patients with deficiency in these factors.

We first studied the role of ATR during CSR and SHM. CSR junctions obtained from ATR deficiency (ATRD) patients showed significantly increased usage of microhomology, but impaired end joining with partially complementary (1-3 bp) DNA ends. The SHM pattern was also altered, with fewer mutations occurring at A but more at T residues, representing a loss of strand bias in targeting A/T pairs within certain hotspots. The function of ATR and ATM in CSR and SHM was also compared. Our data suggest that the role of ATR is partially overlapping with ATM, whereas ATR is also endowed with unique functional properties in the repair processes during CSR and SHM.

We further studied the CSR junctions in Artemis deficient patients. A significantly increased usage of microhomology of ≥10 bp and an absolute absence of blunt-end joining were observed in Sμ-Sα junctions in the patients. However, the Sμ-Sγ junctions obtained from a patient who carried "hypomorphic" mutations appeared to be largely normal in their usage of microhomology, although an unusual type of sequential switching was observed more frequently than expected. These findings suggest that varying modes of CSR junction resolution were used for different S regions, when the function of Artemis is impaired. The altered pattern of CSR junctions also strongly link Artemis to the predominant NHEJ pathway during CSR.

CSR junctions were also studied in B cells from Cernunnos deficient patients. These junctions were characterized by a significantly increased usage of microhomology of ≥10 bp and a significantly decreased usage of "direct end joining". This pattern has previously been observed in B cells deficient for DNA Ligase IV (Lig4), XRCC4, Artemis and ATM, suggesting that Cernunnos is likely to be involved in the DNA Lig4 dependent NHEJ pathway during CSR.

One somatically acquired missense mutation (p.Q227R) was also observed in the Cernunnos encoding gene in a germinal B cell like (GCB) diffuse large B cell lymphoma (DLBCL) sample. Two types of translocations (*IGH/BCL2* and *MYC/IGH*) were detected in this tumor sample and one of the switch γ regions appeared to be disrupted during translocation. Clonal-like, dynamic IgA switching activities were also observed, suggesting a link between defects in the Cernunnos dependent NHEJ pathway and aberrant CSR/switch translocations during the development of B cell malignancies.

Mutations in *Mre11* and *NBS1* gene can cause Ataxia-telangiectasia-like disorder (ATLD) and Nijmegen breakage syndrome (NBS) respectively. SHM patterns in cells from these patients were furthermore analyzed. The frequency and distribution of mutations obtained from both patient groups were largely similar to that observed in controls. The mutation pattern from ATLD patients was only slightly changed, with a small increase of the frequency of A to C transversions, suggesting that Mre11 is unlikely to be the major nuclease involved in cleavage of the abasic sites during SHM. The mutation pattern from NBS patients was however, altered with a significantly increased number of G transversions (G→C and G→T), which mainly occurred in AID and/or SHM hotspot motifs. NBS1 might thus have a specific role in regulating the strand-biased repair during phase Ib mutagenesis.

ISBN 978-91-7457-521-7