

Frequent Loss of the Immunoglobulin Heavy Chain Gene Occurs in FANCI-deficient DT40 Cell Line

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Objective: Fanconi anemia (FA) gene FANCI (also called BRIP1/BACH1) is a DEAH helicase that interacts with the BRCT domain of BRCA1. Fanci may play an important role to prevent genomic instability, since human FANCI is known as breast cancer susceptibility gene, and FANCI homolog mutant in *C. elegans* (dog-1, for deletion of guanine-rich DNA) displays frequent deletions that start around the 3' end of polyguanine tracts in the genome (Cheung *et al.*, *Nat. Genet.* 2002). Since no apparent defect in I-SceI-initiated homologous recombination (HR) assay was observed in FANCI-deficient DT40 cell line (*fanci*) in previous studies (Bridge *et al.*, *Nat. Genet.* 2005; our unpublished data), it was proposed that FANCI does not contribute to HR. DT40 cell line provides an opportunity to investigate a physiological HR process, since it continues to diversify immunoglobulin (Ig) variable (V) genes through gene conversion, a process that requires (1) activation-induced cytidine deaminase (AID), which initiates DNA lesions in Ig V gene, (2) HR repair factors (e.g. BRCA2), (3) the upstream pseudogene segments as HR templates, (4) active transcription that occurs only in rearranged allele. We noticed frequent and spontaneous loss of Ig heavy chain (IgH) gene in *fanci* cell line, and thus wished to understand the mechanism of this phenomenon.

Methods: We ourselves have generated *fanci* cells by gene targeting. To quantitate the rate of sIgM loss, 24 or more clones of sIgM+ cells were maintained for 3 weeks and analyzed their sIgM expression by Flow cytometry. The IgH loci in magnetic beads-sorted sIgM-negative cells were analyzed by PCR, Southern blotting, or fluorescent *in situ* hybridization (FISH).

Results: The frequent IgH loss occurred only in *fanci* but not in *fance* or *fancd2* cells, and could be suppressed by expression of wild type but not helicase-dead FANCI. Neither unrearranged heavy chain nor light chain genes were affected. Southern blot and FISH analyses indicated that the allelic loss was not due to translocation, but involves deletion of a large undefined region encompassing at least ~14 kb of genomic fragment extending from the upstream pseudogene region to the second exon of constant region (C_μ). The IgH loss was dependent on AID gene expression, and was also largely abrogated by BRCA2 heterozygosity. Although the pseudogene region has been poorly characterized, limited information indicates that it contains highly G/C-rich sequences.

Conclusions: Fanci inactivation causes genome instability leading to frequent IgH loss that likely occurs during Ig gene conversion in DT40 cells. We propose FANCI has some function in HR reactions by acting on recombination intermediates in a sequence-specific manner.

Translational Applicability: Elucidation of the mechanism of genome instability provoked by FANCI dysfunction is important for understanding why FA patients are predisposed to cancer.