Switching on Chromosomal Translocations

Almudena R. Ramiro,[1] Michel C. Nussenzweig,[2,3] and André Nussenzweig[4]

[1]DNA Hypermutation Group, Spanish National Cancer Center, Madrid, Spain; [2]Laboratory of Molecular Immunology, The Rockefeller University; [3]Howard Hughes Medical Institute, New York, New York; and [4]Experimental Immunology Branch, National Cancer Institute, NIH, Bethesda, Maryland

Abstract

Activation-induced deaminase initiates three different antibody diversification reactions: class switch recombination, somatic hypermutation (SHM), and gene conversion. We have shown that, in addition to antibody diversification, activation-induced deaminase can also initiate Burkitt's lymphoma–like c-myc/IgH translocations. However, distinct DNA damage- and oncogene-induced checkpoints operate in B cells to produce a normal intrachromosomal class switch recombination event or an aberrant interchromosomal fusion. These findings open the way to study the molecular pathways taking place at the early stages of malignant transformation. (Cancer Res 2006; 66(16): 7837-9)

B cells are especially prone to undergo malignant transformation. B-cell lymphomas represent 95% of all the lymphomas diagnosed in the western world, many of which are derived from mature B cells that were actively undergoing an immune response in structures called germinal centers (1). A hallmark of mature B-cell lymphomas is the presence of reciprocal chromosomal translocations that juxtapose loci encoding the immunoglobulin (Ig) genes and a proto-oncogene. As a result of the fusion, the oncogene is under control of the Ig enhancer, leading to its deregulated expression (1). An additional feature of B-cell lymphomas is the acquisition of mutations in tumor suppressor genes, such as p53 and ATM. However, whether loss of tumor suppressor genes can contribute early in tumor progression remains unclear.

After antigen encounter, mature B cells engage in the germinal center reaction to cause a secondary antibody repertoire that is key to the development of an efficient immune response. This antibody refinement is carried out through two independent mechanisms, somatic hypermutation (SHM) and class switch recombination (CSR; ref. 2). SHM involves the introduction of nucleotide substitutions within the antigen-binding, variable region of the antibody, enabling an increase in affinity for antigen. CSR is a region-specific recombination reaction that substitutes the Ig constant region for one of a collection of downstream constant regions that provides the antibody with new effector functions. For >20 years, malfunctioning of either of these two germinal center reactions has been considered as the plausible trigger of the translocations usually associated with mature B-cell lymphomas. However, until recently, a genetic experimental approach to this issue was not feasible.

Requests for reprints: Almudena R. Ramiro, DNA Hypermutation Group, Spanish National Cancer Center, Madrid 28029, Spain. Phone: 34-91-732-8055; Fax: 34-917-8033; E-mail: arodriguezr@cnio.es.

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Both SHM and CSR are initiated by the activity of activation-induced deaminase (AID), an enzyme selectively expressed in germinal center B cells (3). Mice deficient in AID cannot perform CSR or SHM (4), and mutations in the AID gene are associated with hyper-IgM type 2 immunodeficiency in humans (5). AID initiates SHM and CSR by deaminating cytidine nucleotides in the variable and switch regions, respectively, giving rise to U:G mismatches in the Ig locus. U:G mismatches are subsequently processed by uracil DNA glycosylase or mismatch repair enzymes, which generate either a mutation (in the case of SHM) or DNA double-strand breaks that are rejoined by nonhomologous end joining (in the case of CSR; ref. 6).

The identification of AID as a mutagenic factor that is expressed during germinal center reactions brought into picture a testable candidate to account for the translocations found in B-cell lymphomas. This possibility was especially attractive in the case of CSR, as DNA double-strand breaks intermediates generated in this reaction are also potent substrates for translocations. To test this idea, we made use of mice transgenic for interleukin-6 that develop lymphoid hyperplasia and spontaneous translocations of the c-myc proto-oncogene to the switch region of the Ig locus (7). We showed that these translocations were not found in $AID^{-/-}$IL6tg mice, therefore providing genetic evidence for the requirement of AID for c-myc/IgH translocations *in vivo* (8). However, transgenic for interleukin-6 lymphoid hyperplasia is a long latency disease and there remained the possibility that AID was not directly required to generate the translocation itself but rather to promote secondary mutations that would allow the outgrowth or survival of B cells already harboring AID-independent translocations (9).

We reasoned that if c-myc/IgH translocations were truly a byproduct of CSR, then we might be able to generate them *in vitro* under conditions where CSR is induced with a high efficiency. Indeed, overexpression of AID in $AID^{-/-}$ spleen B cells activated to switch *in vitro* promoted the appearance of c-myc/IgH translocations within 24 hours (10). Moreover, the generation of c-myc/IgH translocations was strictly dependent both on AID deaminase activity and on uracil DNA glycosylase activity. Therefore, CSR, c-myc/IgH translocations, and IgH-associated breaks are initiated by a common mechanism involving DNA deamination and uracil removal. Interestingly, we found that Ku80, one of the nonhomologous end joining components involved in processing DNA breaks during CSR, was not required for the resolution of c-myc/IgH translocations, implying that alternative, nonclassic nonhomologous end joining could be involved in aberrant interchromosomal joining.

Translocations were also found in cells expressing physiologic levels of AID but at a frequency about a thousand times lower than in overexpression conditions. This observation suggested that there could be cellular surveillance mechanisms preventing AID-induced translocations that are simply overwhelmed when AID expression levels increase above a threshold. In search for such protective

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	Unresolved IgH breaks	p53 deficiency	C-myc/IgH translocation frequency
WT	++	No defect	++
53BP1$^{-/-}$	++++	No defect	++++
p53$^{-/-}$	++	+++++	++++++++
ATM$^{-/-}$	++++++	++	++++++++

Cancer Research Reviews

Table 1. Translocation frequency in cells stimulated to undergo class switching may be proportional to the product of unresolved DNA breaks at the IgH and c-myc loci and the impairment in p53 signaling. Note that the frequency of DNA breaks at the c-myc loci is unknown.

pathways, we reasoned that factors involved in normal CSR and the surveillance of DNA damage, such as ATM, H2AX, and 53BP1, might function to prevent DNA breaks from being channeled to translocations. Loss of H2AX and 53BP1 results in a profound impairment of CSR possibly because these proteins are involved in juxtaposing distal switch regions (normally separated by >100 kb) before joining by nonhomologous end joining (11). However, absence of H2AX or 53BP1 did not have a measurable effect on the frequency of c-myc/IgH translocations. In contrast, cells deficient in ATM, with a milder defect on CSR, displayed a dramatic increase in translocation frequency. Therefore, distinct DNA damage sensor pathways operate in B cells to deal with double-strand breaks either leading to a normal CSR event or preventing an aberrant resolution as a translocation.

ATM can detect and respond to DNA double-strand breaks by activating the p53 tumor suppressor. p53 plays a key role in eliminating cells harboring unresolved DNA breaks either through induction of cell cycle checkpoints or apoptosis. We found that this response is also part of the surveillance pathway that prevents the appearance or survival of translocation harboring cells, because B cells deficient in p53 displayed a higher frequency of AID-induced c-myc/IgH translocations. Interestingly, even the loss of a single p53 allele increased the frequency of translocations. In addition to DNA breaks, p53 can be activated through the p19 (ARF) tumor suppressor in response to abnormal mitogenic signals, such as those triggered by c-myc deregulation as a result of chromosomal rearrangements. p19$^{-/-}$ B cells were also found to be more prone to c-myc/IgH translocations, pointing to a complementary protective mechanism that eliminates cells after the translocation has already taken place.

Although the efficiency of CSR is significantly reduced in H2AX$^{-/-}$ and 53BP1$^{-/-}$ mice, p53- and p19-deficient B cells do not display any detectable CSR defect. Therefore, there is no correlation between impairment of the switch recombination reaction and susceptibility to suffer c-myc/IgH translocations. We sought to determine if this susceptibility could be traced to the presence of chromosomal lesions at the IgH locus. By analyzing metaphase spreads using fluorescent *in situ* hybridization with IgH-specific probes, we found that ATM$^{-/-}$ B cells activated to undergo CSR accumulate a high level of chromosomal aberrations. These were nearly equally divided among general chromosomal aberrations and those occurring specifically at the IgH locus. Similarly, 53BP1$^{-/-}$ and H2AX$^{-/-}$ B cells (10, 12) show IgH-associated instability but lower compared with ATM$^{-/-}$ mice that have a milder defect in CSR. In contrast, we found background levels of both general and IgH-associated chromosomal aberrations in p53$^{-/-}$ and p19$^{-/-}$ B cells. Thus, genomic instability per se is insufficient to generate c-myc/IgH translocations.

Because DNA breaks are substrates for translocations, it seems counterintuitive that elevated levels of IgH-associated breaks and defective CSR are not predictive of aberrant chromosomal fusions. Our data suggest that susceptibility to c-myc/IgH translocations may be proportional not only to the frequency of unresolved breaks (at IgH and c-myc loci) but also to the degree of impairment in p53 signaling (Table 1). Although 53BP1$^{-/-}$ B cells are severely defective in CSR and accumulate higher-level IgH breaks than wild-type, p53 signaling is normal in the absence of 53BP1. As a result, most of the cells harboring free, broken ends might be eliminated. p53 deficiency results in high levels of translocations despite low levels of IgH breaks because these recombinagenic ends are efficiently channeled into translocations. ATM$^{-/-}$ mice accumulate elevated levels of IgH breaks and p53 signaling is partially defective, which might synergistically increase the frequency of translocations. This model predicts that absence of p53 in either a 53BP1 or ATM knockout background would further

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enhance c-myc/IgH fusions. Although this has not been tested *in vitro*, it has been established that combined loss of p53 and factors that facilitate Ig switch synapsis (e.g., 53BP1 and H2AX) leads to accelerated appearance of B-cell lymphomas that harbor c-myc/IgH translocations (13, 14).

Mature B-cell lymphomas are the most common lymphoid malignancies in humans and many of these are associated with cytogenetic abnormalities involving Ig loci (1). In Burkitt's lymphoma and diffuse large B-cell lymphoma, one key transforming event seems to be chromosomal translocations involving c-myc; in addition, p53 pathway components (p53, p19, and ATM) are frequently mutated in these tumors. Burkitt's lymphoma and diffuse large B-cell lymphoma are thought to arise from normal B cells in germinal centers, which uniquely express high levels of AID and reduced levels of p53 (15). Our data suggest a mechanism by which mutation or decreased p53 expression contributes early in the pathogenesis of lymphoma by facilitating AID-induced translocations.

Several questions remain. Various proto-oncogenes are engaged in IgH-associated translocations in different mature B-cell lymphomas, some of which have been reported to accumulate AID-like mutations (16). Are these oncogenes actually targeted by AID, and if so, what makes them susceptible to AID activity? Many mature B-cell lymphomas harbor translocations in the Ig light chain loci, which are not subject to CSR. Can SHM-associated DNA lesions also lead to chromosomal translocations? ATM facilitates CSR while preventing chromosome translocations. What distinguishes a double-strand break suitable for switch synapsis from a deleterious one? How are DNA ends joined in a translocation in the absence of Ku80? What pathways downstream of p53 are activated in translocation-positive cells? Is the fate of these cells apoptosis or cell cycle arrest? Further research on these issues will undoubtedly add to the understanding of the early stages of B-cell malignant transformation.

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