Take care of your chromosomes lest cancer takes care of you

The analysis of compound mouse mutants for nonhomologous end-joining DNA double-strand break repair and those deficient for the p53 checkpoint pathway has provided a fascinating look at the carcinogenic consequences of the failure to properly repair DNA damage and to elicit appropriate checkpoints.

In the last few years, the cancer field has gained greater insights into and increased appreciation for the genes and pathways involved in the maintenance of genome integrity. Indeed, it has long been recognized that most, if not all, human cancers display aberrations in chromosome count and structure whose recurrent nature belies a pathogenetic link to cancer development (Lengauer et al., 1998). For certain human cancers, such as those of the hematopoeitic system, there exists ample genetic evidence that certain recurrent chromosomal translocations are diagnostic and causative (Rowley, 2001). While debate continues over the origins of such changes and their precise contributions to carcinogenesis (Marx, 2002), mounting experimental evidence has strengthened the view that periods of genetic instability can propel aspiring cancer cells toward a lethal malignant endpoint.

The path to our current understanding has been illuminated largely through the systematic analysis of mouse models defective for telomere maintenance, DNA repair, and tumor suppressor pathway function (Hakem and Mak, 2001; Maser and DePinho, 2002). These model systems have demonstrated that mice, deficient in the various "caretakers" of the genome, sustain a spectrum of tumors that recapitulate more closely certain cytogenetic aspects of human cancers. Particularly informative have been mouse models harboring deficiencies of components of the nonhomologous end-joining pathway (NHEJ) of DNA double-strand break (DSB) repair (Ferguson and Alt, 2001). Unable to complete the programmed rearrangements initiated by DSBs induced by the RAG-1/2 nuclease, these mutant mouse strains have offered a novel view of the potential consequences of a free DSB—namely, the checkpoints elicited by unrepaired DSBs and the detrimental impact of inappropriate DSB repair. Mice deficient for p53 checkpoint function alone sustain high rates of T cell lymphomas and sarcomas, yet remarkably these tumors lack characteristic chromosome structural anomalies and exhibit only modest levels of aneuploidy. In contrast, mice with dual impairments in NHEJ and p53 function succumb to highly aggressive pro-B cell lymphomas possessing signature chromosomal rearrangements whose anatomy points to a defective V(D)J recombination process (Difilippantonio et al., 2002; Gladdy et al., 2003 [this issue

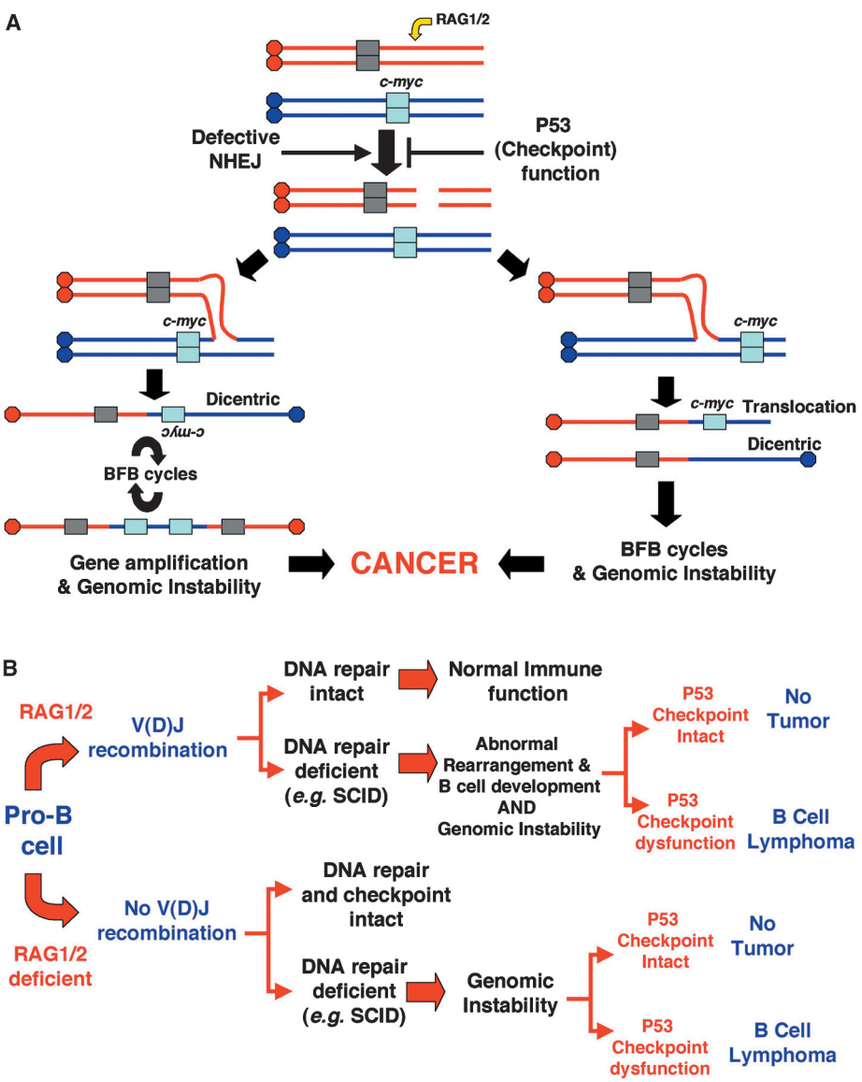

Figure 1. Creating a cancerous genome in the absence of NHEJ and p53, and pathways to pro-B cell lymphoma in mice

A: In cells lacking p53 checkpoint function, the failure of NHEJ to properly repair the DNA DSB created by RAG-induced cleavage at IgH leads to the formation of aberrant chromosomes (translocations and dicentrics). In turn, these can result in *c-myc* amplification, BFB cycles, and further genomic instability, creating the genetic recipe for pro-B cell lymphoma in mice.

B: Once initiated, improper completion of V(D)J recombination in mice lacking NHEJ and p53 function results in a path that leads to pro-B cell lymphoma. Alternatively, in the proper genetic context, generalized genomic instability in NHEJ-p53 double mutants may also lead to a path to pro-B cell lymphoma.

of *Cancer Cell*]; Vanasse et al., 1999; Zhu et al., 2002).

These compound NHEJ p53 mutant model systems have offered a penetrating view of the cytogenetic and oncogenic consequences of an introduced DNA DSB (created by RAG nuclease) and its aberrant repair in a setting of attenuated DNA damage checkpoints. Among these studies, several common themes have emerged. In each case, for mice lacking p53 and either Ku70 or Ku80 (Difilippantonio et al., 2002), XRCC4 or LIG4 (Zhu et al., 2002), or carrying a hypomorphic DNA-PK_{cs} "SCID" mutation (DNA-PK^{SCID}) (Gladdy et al., 2003; Vanasse et al., 1999), there emerges lymphomas bearing clonal and recurrent chromosomal rearrangements. The advent of high-resolution cytogenetic technologies, such as spectral karyotyping and array-comparative genomic hybridization, has allowed the field to molecularly define these rearrangements and thereby infer the mechanism of repair that produced them, gaining insight into the molecular forces guiding cancer cell evolution. In nearly every lymphoma, rearrangements appear to initiate from the site of RAG-induced cleavage in the immunoglobulin heavy chain locus (IgH) on chromosome 12, and subsequently involved the *c-myc* locus on chromosome 15 (Figure 1A). Repair of the RAG-induced DSB and generation of the (12;15) translocation appear to have resulted from a type of end repair utilizing short homologies (Difilippantonio et al., 2002; Gladdy et al., 2003; Zhu et al., 2002). In addition, evidence suggests that dicentric formation and ensuing bridge-fusion-breakage (BFB) cycles are involved in creating an amplified *c-myc* locus and more complex chromosomal rearrangements (Figure 1A) (Difilippantonio et al., 2002; Gladdy et al., 2003; Zhu et al., 2002). The reason that *c-myc* is so frequently involved is unclear, but may reflect the presence of fragile sites, cryptic sequences recognized by RAG-1/2, and/or the chance occurrence of a DSB generated by the breakage of dicentrics. With regard to the latter, it is interesting to note that certain NHEJ deficiencies are associated with decreased telomere capping function and the production of telomeric end-to-end fusions (Bailey et al., 1999; d'Adda di Fagagna et al., 2001) that would in turn set the stage for BFB-induced DSBs throughout the genome. In this scenario, the critical role of c-Myc dysfunction in lymphomagenesis would presumably lead to the selection of rare cells in the population that harbor an amplified *c-myc* locus. Combined, these results point to the central importance of DSBs in genomic instability, particularly aberrations in chromosome structure, which can fuel pro-B cell lymphomagenesis.

More recent work has taken a step further by assessing the role of RAG in initiating the DSBs and specific chromosome abnormalities that drive pro-B cell tumors (Difilippantonio et al., 2002; Gladdy et al., 2003; Vanasse et al., 1999; Zhu et al., 2002), and unexpectedly, some different outcomes were observed. The absence of RAG-1/2 prevents the initiation of V(D)J recombination due to the failure to create the initial cleavage at immune loci; hence, the lack of RAG-1/2 might be expected to prevent pro-B cell lymphomas in mice deficient for both NHEJ and p53 function. This was tested previously (Difilippantonio et al., 2002; Vanasse et al., 1999; Zhu et al., 2002) and in the study in this issue of *Cancer Cell* (Gladdy et al., 2003) by breeding RAG-2 deficiency into the various NHEJ/p53 mutant strains. Not surprisingly, pro-B cell lymphomas with the recurrent (t12;15) translocations were suppressed; however, beyond this common finding, interesting differences were obtained among the various studies. Mice mutant for Xrcc4-p53 combined with RAG-2 deficiency died young, before they would be expected to develop the p53-associated T cell lymphoma observed in NHEJ-proficient mice (Zhu et al., 2002), whereas Ku80-p53 mutant mice with RAG-2 deficiency did develop T cell lymphomas at a later age, as do their Ku80 wild-type p53-deficient counterparts (Difilippantonio et al., 2002). Vanasse et al. (1999) observed that DNA-PK^{SCID}-p53-RAG-2 mutant mice behaved similarly, developing late T cell rather than early B cell lymphomas. Whereas this apparent difference in cancer phenotype may reflect the shorter lifespan of the triple mutant Xrcc4-p53-RAG-2 mice compared to the DNA-PK^{SCID}-p53-RAG-2 mutant mice, we are tempted to speculate that this distinction could reflect in part the differential impact of the various NHEJ deficiencies on telomere maintenance (d'Adda di Fagagna et al., 2001). In striking contrast, Gladdy et al. (2003) observed no reduction in either incidence or latency of pro-B cell lymphomas in their DNA-PK^{SCID}-p53-RAG-2 mutant mice compared to DNA-PK^{SCID}-p53 mutant controls. Whereas lymphomas of the DNA-PK^{SCID}-p53 mutant cohort harbored the characteristic RAG-induced rearrangements, this tumor was suppressed in the triple mutant background. In its absence, however, a different type of pro-B cell lymphoma was revealed which lacked the (t12;15) translocation, possessed different translocations, and had different biological properties. In the latter context, the Gladdy et al. DNA-PK^{SCID}-p53-RAG-2 mutant pro-B cell lymphomas were unique in their capacity to spread to the meninges—the membranes enveloping the central nervous system (Gladdy et al., 2003). This interesting and novel experimental outcome suggests that generalized genomic instability associated with NHEJ deficiency in the appropriate context can cooperate with p53 checkpoint deficiency to create lesions capable of endowing pro-B cell lymphomas with the capacity to invade and reside in the meningial membranes—although, as discussed below, additional factors may be operative. Nonetheless, expression of c-Myc was elevated compared to normal lymphoid cells, pointing to its central importance in lymphoma pathogenesis and the capacity of c-Myc to be dysregulated via multiple mechanisms.

What are we to make of the differences in the pro-B cell tumor phenotype arising in the Gladdy et al. triple mutant DNA-PK^{SCID}-p53-RAG-2 cohort compared to the other studies? As far as can be discerned, the mutant alleles employed were identical: RAG-2, DNA-PK_{cs} (SCID), and p53 (regardless, two different alleles of p53 have been shown to behave similarly [Difilippantonio et al., 2002]). Is it possible that the phenotypic differences relate to various modifiers present in these heavily mixed genetic strains? The fact that NHEJ and p53 deficiencies result in a near-universal pro-B cell lymphoma condition suggests that these combined mutations drive this tumor type regardless of genetic background in RAG-proficient animals. In the Gladdy et al. study (Gladdy et al., 2003), one might assume that a modifier difference may influence the phenotypic outcome of the triple mutant mice, although this seems unlikely given the heavily mixed composition of the large experimental cohorts examined. Nonetheless, a specific genetic modifier in the Gladdy et al. triple mutant cohort cannot be discounted, nor can we exclude the modu-

lating effects of environmental and dietary factors. Despite these differences, it appears that DSBs, whether induced by the RAG nuclease or by the random BFB process enabled by NHEJ/p53 deficiency, can lead to pro-B cell lymphomas (Figure 1B). These unresolved issues should motivate efforts to identify the genetic and epigenetic factors that endow lymphoma cells with the capacity to hone to meningial membranes—an occurrence that is not uncommon in the lymphoma clinic.

Are there any unifying themes associated with these studies? For one, it is clear that NHEJ deficiency (as well as other DNA repair defects) results in genomic instability that can provoke carcinogenesis. This becomes abundantly clear when programmed DNA DSBs are initiated in the absence of NHEJ in the lymphoid compartment. Although the *c-myc* rearrangements found in the NHEJ/p53 double mutant murine tumors are not identical to those typically found in human cancers (Rowley, 2001), they are informative nonetheless with respect to identifying mechanisms that contribute to the chromosomal aberrations encountered in a wide variety of human lymphoid and solid tumors. Gene amplification (and deletion) can contribute to a wide spectrum of tumor types, and the genetic experiments described here and previous cell culture studies (Pipiras et al., 1998) indicate that DSBs are crucial initiators of these events. Second, the p53 checkpoint mechanism in response to aberrant DNA DSBs has once again shown its central importance and an additional basis for why its loss of function can participate in the development of so many tumor types. In the lymphoid compartment, its function is to force aberrant cells into apoptosis, rather than to allow alternative pathways of DNA repair (Figure 1B). The link between DSBs, p53 status, dicentric formation, and increased cancer—particularly epithelial cancers—was first forged in mice deficient for telomere function (Artandi et al., 2000; Chin et al., 1999), in which p53 loss provides a permissive context for the increased formation of chromosomal fusions and recurrent rearrangements via BFB cycles that fuel cancer initiation. Analogously, in the NHEJ mutants, p53's absence enables existing DNA damage to be replicated and create the aberrant chromosomes found in tumors. Lastly, this body of work continues to validate the use of genetically engineered mice as models to elucidate mechanisms governing chromosome stability and to uncover novel genetic pathways that promote or prevent human cancer.

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