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Gary J. Vanasse,1,4 James Halbrook,2 Sushma Thomas,3 Abigail Burgess,4 Merl F. Hoekstra,2 Christine M. Disteche,3 and Dennis M. Willerford1,4

1Department of Medicine, University of Washington, Seattle, Washington 98195, USA
2ICOS Corporation, Bothell, Washington 98021, USA
3Department of Pathology, University of Washington, Seattle, Washington 98195, USA
4Puget Sound Blood Center, Seattle, Washington 98104, USA

Address correspondence to: Dennis M. Willerford, Puget Sound Blood Center, 921 Terry Avenue, Seattle, Washington 98104, USA.
Phone: (206) 292-7077; Fax: (206) 343-1776; E-mail: dwiller@u.washington.edu.

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Chromosome translocations involving antigen receptor loci are a genetic hallmark of non-Hodgkin’s lymphomas in humans. Most commonly, these translocations result in juxtaposition of the immunoglobulin heavy-chain (IgH) locus with one of several cellular proto-oncogenes, leading to deregulated oncogene expression. The V(D)J recombinase, which mediates physiologic rearrangements of antigen receptor genes, may play a mechanistic role in some lymphoma translocations, although evidence is indirect. A high incidence of B-lineage lymphomas has been observed in mice with severe combined immunodeficiency (SCID) and p53-null mutations. We show that these tumors are characteristic of the pro–B-cell stage of development and that they harbor recurrent translocations involving chromosomes 12 and 15. Fluorescence in situ hybridization (FISH) shows retention of IgH sequences on the derivative chromosome 12, implying that breakpoints involve the IgH locus. Pro–B-cell lymphomas were suppressed in SCID p53–/– mice by a Rag-2–null mutation, demonstrating that DNA breaks generated during V(D)J recombination are required for oncogenic transformation, and suggesting that t(12;15) arise during attempted IgH rearrangement in pro-B cells. These studies indicate that the oncogenic potential inherent in antigen receptor diversification is controlled in vivo by efficient rejoining of DNA ends generated during V(D)J recombination and an intact cellular response to DNA damage.


Introduction

Chromosome translocations involving antigen receptor genes represent a central genetic abnormality in the pathogenesis of lymphoid malignancies in humans, particularly the non-Hodgkin’s lymphomas (1–4). Transposition of a cellular proto-oncogene in proximity to the regulatory elements of antigen receptor loci leads to deregulated oncogene expression. The V(D)J recombinase, which mediates physiologic rearrangements of antigen receptor genes, may play a mechanistic role in some lymphoma translocations, although evidence is indirect. A high incidence of B-lineage lymphomas has been observed in mice with severe combined immunodeficiency (SCID) and p53-null mutations. We show that these tumors are characteristic of the pro–B-cell stage of development and that they harbor recurrent translocations involving chromosomes 12 and 15. Fluorescence in situ hybridization (FISH) shows retention of IgH sequences on the derivative chromosome 12, implying that breakpoints involve the IgH locus. Pro–B-cell lymphomas were suppressed in SCID p53–/– mice by a Rag-2–null mutation, demonstrating that DNA breaks generated during V(D)J recombination are required for oncogenic transformation, and suggesting that t(12;15) arise during attempted IgH rearrangement in pro-B cells. These studies indicate that the oncogenic potential inherent in antigen receptor diversification is controlled in vivo by efficient rejoining of DNA ends generated during V(D)J recombination and an intact cellular response to DNA damage.

Much has been learned in the past few years regarding the mechanism of V(D)J recombination. DNA scission is catalyzed by the Rag-1 and Rag-2 proteins at recognition signals (RSS) bordering antigen receptor variable-region gene segments, creating blunt signal ends and sealed hairpins at coding ends (14–17). Mice and humans with induced null mutations of Rag-1 or Rag-2 are unable to initiate rearrangement of endogenous Ig or TCR loci and consequently lack mature B or T cells (18–20). Rejoining of coding ends requires the DNA-dependent protein kinase (DNA-PK) complex, including the catalytic subunit (DNA-PKcs), Ku70 and Ku86, whereas signal ends are resolved by a distinct pathway, also involving the Ku proteins (reviewed in refs. 6, 21). Severe combined immunodeficiency (SCID) mice, which have a mutation affecting the DNA-PKcs, are capable of initiating Rag-mediated DNA cleavage but are severely limited in their ability to efficiently rejoin coding ends. As a result, there is a near-complete arrest of T- and B-cell development at an early progenitor stage, with accumulation of unresolved DNA breaks at coding ends (22–26). The p53 tumor suppressor gene is mutated in a wide variety of human cancers and plays an important role in the cellular response to DNA damage. DNA breaks lead to rapid upregulation of p53 expression, which leads to 2 major cellular effects: arrest of the cell cycle at the G1
phase and induction of apoptosis (27–29). Mice lacking p53 are susceptible to several types of malignancies; of these, up to two thirds are lymphomas, which are predominantly localized thymic tumors (30, 31). SCID mice are also susceptible to thymic lymphomas, which may occur in up to 15% of animals (23). Mice with combined SCID and p53-null mutations develop disseminated B-cell lymphomas with an incidence approaching 100%; these lymphomas occur at a younger age than do lymphoid tumors in either parental strain (31–33). In this report we show that pro–B-cell lymphomas in SCID p53+/– mice harbor recurrent translocations involving the telomere of chromosome 12, at or near the IgH locus, and that tumor development requires initiation of V(D)J recombination. This genetic pathway to recurrent lymphoma translocations indicates that mitigation of the oncogenic potential inherent in antigen receptor gene rearrangement requires efficient rejoining of cleaved DNA and an intact cellular response to DNA damage.

Methods

Mice. SCID p53+/– mice (ICR and C57Bl/6 backgrounds; Taconic Farms, Germantown, New York, USA) were intercrossed to generate SCID p53+/– mice. Rag-2+/– mice (C57Bl/6 and 129 backgrounds; obtained from Frederick Alt, Children’s Hospital, Boston, Massachusetts, USA) were crossed with SCID p53+/– mice, and offspring were bred to generate the following 3 experimental cohorts: p53–/– (SCID++, Rag-2+/–, or Rag-2–/–); SCID p53+/– (Rag-2+/– or Rag-2–/–); and SCID p53+/– Rag-2–/–. The genotype of all mice was confirmed by PCR for each allele. Mice were housed under specific pathogen-free conditions and sacrificed upon recognition of tumor masses or ill health, which marked the endpoint for calculating tumor incidence. Kaplan-Meier analysis was performed using the SPSS software package, and separate analyses were performed for mortality due to disseminated pro–B-cell lymphomas and for all tumors. Statistical analysis of survival differences was performed using the Wilcoxon log rank test.

Analysis of tumor phenotype. Tumor tissue was fixed in formalin for standard histology. For flow cytometry, cell suspensions were stained using FITC-, phycoerythrin-, biotin-, or allophycocyanin-conjugated antibodies to the indicated markers, with secondary staining by streptavidin-CyChrom (PharMingen, San Diego, California, USA). Analysis was performed using a dual-laser FACScalibur (Becton Dickinson Immunocytometry Systems, San Jose, California, USA). Cytosin preparations from primary tumors were fixed in ice-cold acetone and stained using horseradish peroxidase-conjugated goat anti-mouse IgM, µ-chain specific, followed by colorimetric detection using 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, Missouri, USA) according to the manufacturer’s protocol. Controls included normal mouse spleen cells, murine cell lines p5242 (pro-T cell), and WEHI 231.1 (immature B cell).

Table 1

<table>
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<th>Number</th>
<th>Genotype</th>
<th>JH</th>
<th>JH</th>
<th>Gμ</th>
<th>Gμ</th>
<th>Cδ</th>
<th>Cδ</th>
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<td>G</td>
<td>G</td>
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<td>Del</td>
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</tr>
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<td>Del</td>
<td>G</td>
<td>R</td>
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<td>G</td>
<td>G</td>
<td>G</td>
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<td></td>
</tr>
</tbody>
</table>

Genomic digests using EcoRI, BamHI, and BglII were analyzed using the indicated probes, except for Cδ, in which EcoRI and BamHI digests were analyzed. For each probe, the status of both alleles is indicated. G, germ line; R, rearranged; Del, deleted. For germ-line bands in which the status of the second allele could not be determined (i.e., either germ line or deleted), the second allele is denoted by an asterisk.

Figure 1

Flow cytometric analysis of primary tumor cells from lymphomas arising in p53–/– and SCID p53–/– mice. The majority of p53–/– tumors were thymic lymphomas with T-cell markers (top row, representative of 5 tumors), whereas 1 peripheral B-cell lymphoma expressing IgM was observed (middle row). SCID p53–/– lymphomas (bottom row, representative of 24 tumors) expressed B-lineage markers but lacked IgM expression. Single-cell suspensions from primary tumors were stained with antibodies to the markers indicated. Fluorescence intensity is expressed on a logarithmic scale. Control (unstained) plots are shown in broken lines.

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Results

SCID p53−/− lymphomas have a pro-B-cell phenotype. In agreement with prior reports in the literature (31–33), we found that 24 of 24 SCID p53−/− mice developed lymphomas at 6–13 weeks of age, with lymphomas observed in 6 of 15 p53−/− animals at later times. SCID mice had a significantly lower tumor incidence. Lymphomas in p53−/− mice included 5 thymic lymphomas and 1 localized neck mass. In contrast, SCID p53−/− tumors were disseminated throughout lymphoid tissue and other organs, including kidney and liver. Peripheral blood films demonstrated that lymphomas in 6 of 6 SCID p53−/− mice were associated with frank leukemia, a finding that was absent in 2 of 2 p53−/− lymphomas studied. Histologic examination of SCID p53−/− tumor tissue revealed near-total replacement of normal lymphoid architecture with high-grade lymphoma.

Flow cytometric analysis of 24 SCID p53−/− lymphomas revealed expression of B-cell markers B220 and CD19, but not T-cell markers CD4, CD8, or CD90 (Figure 1). These revealed expression of B-cell markers B220 and CD19, but revealed near-total replacement of normal lymphoid tissue and other organs, including kidney and liver. Peripheral blood films demonstrated that lymphomas in 6 of 6 SCID p53−/− mice were associated with frank leukemia, a finding that was absent in 2 of 2 p53−/− lymphomas studied. Histologic examination of SCID p53−/− tumor tissue revealed near-total replacement of normal lymphoid architecture with high-grade lymphoma.

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lacked t(12;15), as determined by dual-color FISH analysis.

The IgH locus extends to the telomere on human chromosome 14 (41) and is similarly positioned on mouse chromosome 12, although continuity with the telomere has not yet been shown. By G-band analysis, the chromosome 12 breakpoint is consistent with the murine IgH complex. To further localize the breakpoint region, primary tumor metaphase spreads were hybridized with a BAC probe containing the 3′ part of the IgH locus, extending from the proximal VH segments to include Cα. In 4 of 6 tumors analyzed, the IgH BAC signal was retained on the derivative chromosome 12 (der12; Figure 3b and Table 2), implying that the breakpoint was within the IgH locus. Lack of an IgH signal in 2 t(12;15) tumors could indicate that the breakpoint is located toward the 3′ end of the IgH locus (where the BAC probe may have diminished sensitivity) or centromeric to the probe. We did not detect a reciprocal t(15;12), either with chromosome paints or with the IgH BAC probe. Taken together, these data show that SCID and p53-null mutations define a genetic pathway to pro–B-cell lymphomas that carry recurrent translocations involving chromosome 12, probably within the IgH locus.

Review of the genetic map of chromosome 15 suggested c-myc as a candidate oncogene for involvement in t(12;15) translocation. The position of c-myc in t(12;15) pro–B-cell lymphomas was analyzed using dual-color FISH with probes for c-myc and the centromeric region of chromosome 12 (Figure 3c). Whereas c-myc signals were readily recognized on chromosome 15, they were absent from the der12 chromosome. Similar results were obtained in 4 additional t(12;15) tumors, indicating that c-myc is not deregulated directly by the t(12;15) translocations, and suggesting the involvement of another oncogene on chromosome 15.

Initiation of V(D)J recombination is required for t(12;15) pro–B-cell lymphomas in SCID p53–/– mice. Predisposition to t(12;15) pro–B-cell lymphomas in SCID p53–/– mice involves a lesion in the V(D)J recombinase, suggesting that translocations may arise as a result of aberrant IgH rearrangement in pro-B cells. By introducing a Rag-2–null mutation into the SCID p53–/– strain, we tested whether initiation of V(D)J recombination was a required step in oncogenesis. To exclude background effects, 3 cohorts of mice were derived from this interbreeding, including p53–/– (SCIDwt/wt, Rag-2+/–, or Rag-2+/+; n = 13), SCID p53–/– (Rag-2+/– or Rag-2+/+; n = 17), and SCID p53+/– Rag-2–/– (n = 14). Mice were observed for tumor development, and tumor phenotype was characterized by flow cytometry as described above. Two separate analyses were performed using survival data for each cohort, considering mortality due to disseminated pro–B-cell lymphoma as well as mortality from all tumor types (Figure 4).

Consistent with previous reports and our earlier experiments, lymphomas developed in 100% of SCID p53–/– mice at a median of 8 weeks. All of these tumors were disseminated B220+CD19+IgM– lymphomas. In the p53–/– cohort, no disseminated pro–B-cell lymphomas were observed during a period of follow-up ranging from 6 to 33 weeks, although 1 animal developed a localized enlargement of a cervical lymph node containing a mature B-cell tumor (B220+CD19+IgM+). In the SCID

Figure 3
FISH analysis of representative SCID p53–/– primary tumor metaphase spreads. (a) Whole-chromosome paints specific for chromosomes 12 (red) and 15 (green). Red arrow shows t(12;15), which was present in the majority of metaphases. Several tumors, including this one, demonstrated a large chromosome 15, possibly representing a duplication or amplification event. (b) IgH BAC probe. Hybridization is seen at the telomere on the normal chromosome 12 (red arrow) and at the breakpoint region on der12 (yellow arrow). Retention of the BAC signal on der12 indicates that the breakpoint lies within or telomeric to the region spanned by the probe. (c) Dual-color FISH analysis using chromosome 12 centromeric-region probe (red arrow) and c-myc probe (green arrow), demonstrating absence of c-myc on the der12 chromosome.
p53\(^{-/-}\) Rag-2\(^{-/-}\) cohort, which was observed for a period ranging from 6 to 22 weeks, no disseminated B-lineage lymphomas were identified. These differences were statistically significant \((P < 0.001)\). Analysis of overall tumor mortality demonstrated that the SCID p53\(^{-/-}\) cohort had the shortest median survival (8 weeks), whereas the median survival of the p53\(^{-/-}\) and SCID p53\(^{-/-}\) Rag-2\(^{-/-}\) cohorts was greater (23 and 18 weeks, respectively). Statistical comparisons indicated that the overall survival of the SCID p53\(^{-/-}\) cohort was significantly less than either of the other groups of mice \((P < 0.001)\), whereas survival differences between the p53\(^{-/-}\) and SCID p53\(^{-/-}\) Rag-2\(^{-/-}\) cohorts were not statistically significant \((P > 0.40)\).

The occurrence of localized thymic lymphomas was similar in the p53\(^{-/-}\) and SCID p53\(^{-/-}\) Rag-2\(^{-/-}\) cohorts (Table 3). Phenotypically, the majority of SCID p53\(^{-/-}\) Rag-2\(^{-/-}\) lymphomas expressed CD90, CD4, and CD8, and lacked TCR-\(\alpha\)\(\beta\), CD3, and B-cell markers. One thymic tumor expressed CD90 as well as the B-cell markers B220 and CD19. Sarcomas were also seen in both the p53\(^{-/-}\) and SCID p53\(^{-/-}\) Rag-2\(^{-/-}\) mice. Thymic lymphomas and sarcomas were not observed in SCID p53\(^{-/-}\) mice, likely reflecting the early mortality from disseminated pro–B-cell tumors (Figure 4b). These data indicate that initiation of V(D)J recombination is a required element in the oncogenic pathway leading to disseminated t(12;15) pro–B-cell lymphomas in SCID p53\(^{-/-}\) mice, but not to the thymic lymphomas and sarcomas characteristic of the p53\(^{-/-}\) background.

**Discussion**

Chromosome translocations involving antigen receptor genes represent a common pathway for lymphoid oncogenesis and likely represent an early and critical genetic change in the evolution of these malignancies (1, 3, 4). The hypothesis that these translocations involve V(D)J recombination has been inferred from the sequences of cloned breakpoints. The evidence appears most consistent in the t(14;18) translocations found in most cases of follicular B-cell lymphomas, which juxtapose the Bcl-2 proto-oncogene with 1 of the JH segments, and in t(7;9) of T-cell lymphoblastic lymphoma/leukemia, which involves breaks flanking the D and J segments of the TCR-\(\beta\) gene on chromosome 7 (4, 8, 9, 12, 42). Other features of V(D)J recombination, including cryptic RSS on partner chromosomes and N-nucleotide additions, have also been reported at translocation breakpoints (reviewed in ref. 4). Despite the resemblance of some breakpoints to V(D)J joints, much of the available data on breakpoint sequences do not strictly conform to the attributes of V(D)J recombination, indicating that most translocations do not arise from a normal V(D)J reaction. Nevertheless, the consistent participation of antigen receptor loci in lymphoma translocations suggests that components of the V(D)J recombination may participate in creating the conditions for aberrant chromosome joining.

The recurrent involvement of t(12;15) in pro–B-cell lymphomas arising in SCID p53\(^{-/-}\) mice defines a genetic pathway leading reproducibly to antigen receptor–locus translocations. The fact that a Rag-2\(^{-/-}\) mutation suppressed the development of t(12;15) lymphomas in the SCID p53\(^{-/-}\) background suggests that the translocations occur as a result of aberrant rejoining of IgH loci cleaved during attempted V(D)J recombination at the pro–B-cell stage. An

**Table 3**

Comparison of tumor types developing in 3 cohorts of mice derived from a common background

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>SCID p53(^{-/-}) ((n = 17))</th>
<th>p53(^{-/-}) ((n = 13))</th>
<th>SCID p53(^{-/-}) Rag-2(^{-/-}) ((n = 14))</th>
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<tr>
<td>Disseminated pro–B-cell lymphoma</td>
<td>17 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Thymic lymphoma</td>
<td>0 (0)</td>
<td>5 (38)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Localized IgM(^{+}) B-cell lymphoma</td>
<td>0 (0)</td>
<td>1 (7.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0 (0)</td>
<td>3 (23)</td>
<td>3 (21)</td>
</tr>
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</table>

Lymphoid tumors were classified by flow cytometry, and sarcomas by gross inspection and histology. The number of mice (percent of cohort) developing each tumor type is given.
The hypothesis that cellular responses to DNA damage suppress oncogenic side reactions of the V(D)J recombinase is supported by the association between genetic conditions affecting these pathways and predisposition to lymphoid malignancies. Patients with ataxia-telangiectasia, the Nijmegen breakage syndrome, and Bloom’s syndrome all exhibit abnormal cellular responses to DNA damage and have a high incidence of non-Hodgkin’s lymphomas (47–50). Lymphomas in ataxia-telangiectasia patients are most commonly T-cell derived and involve translocations with TCR loci on chromosomes 7 or 14 (47). This observation, and the fact that translocation-associated lymphomas in SCID p53–/– mice exclusively involved the B-cell lineage, suggests that the spectrum of critical elements for tumor suppression during antigen receptor rearrangement may differ in the various lymphoid tissues. Advances in understanding the cellular functions of lymphoma tumor suppressors may shed more light on this issue.

Targeted genomic instability is an essential process in diversification of the antigen receptor repertoire and occurs in 3 distinct forms in the B-cell lineage. In addition to VDJ recombination, immune responses induce germinal-center B cells to undergo isotype class-switch recombination within the IgH locus, as well as somatic hypermutation of immunoglobulin variable regions. Class-switch recombination has been implicated in generating t(8;14) in nonendemic Burkitt’s lymphoma, juxtaposing switch regions lying upstream of constant-region exons with c-myc. In mice, plasmacytomas induced by mineral oil are associated with t(12;15), placing the c-myc locus on chromosome 15 into the switch segments of 3’ IgH constant-region genes (13, 51, 52). This mechanism is unlikely to be involved in mediating the t(12;15) in SCID p53–/– lymphomas, given that Rag-2 is required for generation of these tumors but is not needed for class switching (53, 54). Recent data indicate that somatic hypermutation also involves DNA breaks and may contribute to some IgH translocations that occur within the variable region (55, 56). It has also been determined that Rag-1 and Rag-2 are reactivated in germinal-center B cells, where immunoglobulin genes may undergo additional V(DJ) recombination events (57–60). The apparent genetic plasticity of immunoglobulin genes in the germinal center correlates with a postulated germinal-center origin for the majority of B-cell lymphomas and suggests that, in many cases, chromosome translocations may arise in the course of immune responses (61, 62).

Genetic instability at antigen receptor loci represents the distinguishing feature of vertebrate immunity and conflicts with the cellular paradigm of preserving genomic integrity. An important question regarding the mechanism of V(DJ) recombination and other forms of antigen receptor revision is how these processes are normally confined to intended targets. Our data suggest that this confinement involves 2 essential components. First, efficient joining of DNA breaks may minimize cellular exposure to free DNA ends. This is likely facilitated by the coupling of Rag-mediated cleavage events and the formation of a synaptic complex that activates rejoining (63). Second, activation of DNA damage-sensing pathways by unresolved DNA breaks may either kill cells or otherwise prevent the activity of nonspecific repair pathways. Genetic factors or environmental exposures that interfere with either of these processes may increase the frequency of chromosome translocations and contribute to the development of lymphomas.

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Disteche and the University of Washington Royalty Research Fund (to D.M. Willerford).