Chromosomal Instability and Supernumerary Centrosomes Represent Precursor Defects in a Mouse Model of T-Cell Lymphoma

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Abstract

A hallmark of carcinogenesis is resistance to cell death. However, recent studies indicate that Bax expression increased apoptosis and promoted oncogenesis. In this study, we hypothesized that Bax promotes tumor formation by increasing chromosomal instability (CIN). Consistent with this hypothesis, spectral karyotype analysis (SKY) of lymphomas derived from Lck-Bax38/1 mice were consistently aneuploid. To determine if CIN precedes tumor formation, quantitative cytogenetic analysis, SKY analysis, and quantitative centrosome staining were done on thymocytes from young prema-lignant mice. Between 6 and 10 weeks of age, thymi from Bax-expressing mice (either p53+/+ or p53−/−) had an increased percentage of aneuploid cells as well as an increase in cells with supernumerary centrosomes. For 3- to 6-week-old mice, Bax expression increased aneuploidy and supernumery centrosomes in p53−/− mice but not in p53+/+ animals. Importantly, both aneuploidy and supernumerary centrosomes were attenuated by Bcl-2. Remarkably, SKY analysis showed multiple independent aneuploid populations in the p53−/− Bax-expressing mice between 3 and 6 weeks of age. These results indicate that oligoclonal aneuploidy and supernumerary centrosomes are early hallmarks of Bax-induced lymphoma formation and support a novel link between the Bcl-2 family and CIN. The data provide an attractive model for the paradoxical effects of the Bcl-2 family on carcinogenesis that have been observed in multiple studies of both humans and mice. [Cancer Res 2007;67(17):8081–8]

Introduction

The Bcl-2 family is comprised of a large number of genes that are critical regulators of apoptosis. Broadly, these genes can be subdivided into two general classes that either promote or inhibit (Bcl-2, Bcl-XL) cell death. Those members that promote cell death can be classified further as either multidomain members (Bax, Bak) or BH3-only members (Bim, Bid) based on the extent of homology to Bcl-2. The Bcl-2 family regulates apoptosis primarily through their effects on the permeabilization of the outer mitochondrial membrane. They control the release of cytochrome c, Smac/Diablo, and other factors that activate the caspase cascade and result in cell death (1).

Subversion of cell death pathways is a hallmark of oncogenesis and is often mediated by overexpression of antiapoptotic Bcl-2 family members. This is best illustrated by Bcl-2 overexpression in the t(14;18) translocation in human follicular B-cell lymphoma and shown further in transgenic mouse models. In addition, a number of studies have shown the loss of proapoptotic factors such as Bax in both human and murine cancers (2).

Despite this dogma, numerous other studies show alterations in Bcl-2 family members that are not consistent with their role in apoptosis. In clinical studies of human breast (3) and colon (4, 5) cancer, patients with tumors that expressed high levels of Bcl-2 showed increased survival. For the proapoptotic family member Bax, high expression was associated with decreased survival in diffuse large B-cell lymphoma (6), an increased incidence of relapse in childhood acute lymphocytic leukemia (7), and a poor outcome in acute myeloid leukemia (8). This paradox has been extended into mouse models where cause and effect can be tested. Bcl-2 expression delayed both hepatocellular carcinoma in c-myc transgenic mice (9) and mammary tumor formation in mice treated with the carcinogen dimethylbenz(a)anthracene (10). For Bax, high expression accelerated lymphoma development in p53+/+ and p53−/− animals (11, 12). Thus, numerous studies in both humans and mice support a role of the Bcl-2 family in cancer that does not correlate with their apoptotic function.

These data suggest the outcome of Bcl-2 family member expression on cancer is complex and could involve regulation of cellular processes other than apoptosis. Previous studies have linked alterations in expression of Bcl-2 family members to alterations in cellular proliferation. Expression of antiapoptotic factors such as Bcl-XL (13) and Bcl-2 (14, 15) have been shown to delay cell cycle entry, whereas expression of proapoptotic Bax accelerated cell cycle entry and increased cellular proliferation (11, 16). It has been proposed that these alterations in cellular proliferation may account for the paradoxical effects the Bcl-2 family has on carcinogenesis. However, other studies suggest that members of this family may have effects on genomic stability. For example, in a murine model of B-cell lymphoma, Bcl-2 expression was able to maintain genomic stability by abrogating the selective pressure(s) needed to lose p53 and prevented the formation of aneuploid cells (17).

Oncogenesis is a multistep process that requires the acquisition of multiple mutations. It has been proposed that cells must acquire genomic instability as an enabling characteristic of tumor cells and is a reflection of the susceptibility and propensity of the genome to acquire mutations (18, 19). Genomic instability is manifested in three major forms: (a) chromosomal instability (CIN), including gains and losses of whole chromosomes (aneuploidy), chromosome translocations, gene amplifications and deletions, and chromo-some breakage; (b) nucleotide instability; and (c) microsatellite instability (MIN; ref. 20). MIN involves expansions and/or deletions of repetitive nucleotide sequences and is associated with defects in mismatch repair (MMR). Of note, patients with hereditary...
non-polyposis colorectal cancer (HNPCC) inherit a single mutation in one of the enzymes involved in MMR. Loss of the normal allele results in MIN and is thought to initiate oncogenesis in these patients (21). Although much is known about the mechanism of MIN, the pathways that result in CIN, a much more prevalent form of genomic instability, are not well understood. Based on analogy with HNPCC, we sought to determine if CIN is an early, initiating event in lymphoma formation following the expression of Bax.

Materials and Methods

Mice. Transgenic Lck-Bax–, Lck-Bcl-2–, and p53–deficient mice were previously described and are genotyped by the PCR method as previously described (11, 12, 22). The tumors that emerged from Lck-Bax38/1 transgenic mice are of lymphoblastic morphology, and they have a high proliferation rate based on cell cycle analysis of propidium iodide–stained nuclei. Previous immunohistochemistry and flow cytometry studies have shown these tumors to be of T-cell origin based on CD3 and Thy1 staining. The tumors observed in these mice were often large and very aggressive with involvement of the spleen, liver, and kidneys.

Cytogenetic analysis. Cytogenetic analysis was done on freshly isolated thymocytes from transgenic mice or thymic lymphomas that were briefly cultured (<1 h) in vitro. Briefly, 100 μg of colchicine (Sigma) in PBS was injected i.p. into mice to enrich for mitotic cells. Approximately 40 min later, the mouse was sacrificed, and the thymus was excised and minced into single cell suspensions followed by RBC lysis as previously described (11). The resulting cells were then spun onto microscope slides using a cytopsin (Cytospin 2, Shandon) and allowed to air dry. The slides were fixed in −20°C methanol for 6 min and permeabilized for 20 min in 0.4% Triton X-100 in PBS. The slides were then blocked in PBS blocking buffer (PBS, 2% glycerol, 5% 1 mol/L NH₄Cl, 5% FBS) for 1 h at room temperature. The slides were then incubated overnight at 4°C with mouse anti-γ-tubulin (Sigma; T 6557) and polyclonal sheep anti-α-tubulin antibodies (Cytoskeleton; ATN02). The slides were then washed extensively in PBS wash buffer (PBS, 0.15% Tween 20, 0.5% NP40). The slides were then blocked in PBS blocking buffer supplemented with 2% normal donkey serum and 2% normal goat serum for 1 h at room temperature. The slides were then incubated for 2 h at room temperature with Alexa-568–conjugated goat anti-mouse immunoglobulin G (IgG) and Alexa-488–conjugated donkey anti-sheep IgG in PBS blocking buffer supplemented with 100 ng/mL 4,6-diamidino-2-phenylindole (DAPI). The slides were then washed extensively in PBS wash buffer and stained with ToPro3 in PBS for 5 min. The slides were then mounted in ProLong Antifade (Molecular Probes-Invitrogen). To determine the percentage of thymocytes harboring supernumerary centrosomes (three or more per cell), the number of centrosomes in at least 200 cells per mouse were counted in a manner blinded to genotype. Data acquisition for the quantitation studies employed an Olympus BX-51 epifluorescent microscope using the γ-tubulin staining as the centrosome marker. For image acquisition, a Bio-Rad MRC 1024 confocal microscope equipped with filters for Alexa-488, Alexa-568, and ToPro3 was employed. Final images were prepared using Image J Software.

Statistical analysis. Analysis of the distribution of aneuploidy percentages and supernumerary centrosomes from the Lck-Bax38/1 transgenic mice showed that the data did not exhibit a normal distribution. Instead, some animals seemed similar to the controls, whereas other mice had markedly increased aneuploidy and/or supernumerary centrosomes. Therefore, the Fisher’s exact test was used to compare the different groups. Based on the p53+/− controls, a cutoff of 10% was used for the analysis of aneuploidy, whereas a 2% cutoff was used for the analysis of supernumerary centrosomes. These cutoff values were determined by the observed frequency of background noise found in the transgene negative control mice in these studies. A two-tailed Student’s t test was used to compare differences in cellularity between groups.

Results

CIN in Lck-Bax38/1 tumors. Previous studies show that Lck-Bax38/1 mice rapidly develop thymic lymphomas in either p53+/+ or p53−/− backgrounds (12). To determine if aneuploidy is present in tumors from Lck-Bax38/1 mice, SKY analysis on tumors isolated from p53+/+ and p53−/− mice was done (Table 1). All tumors analyzed were aneuploid; however, the karyotypic and/or structural aberrations observed were markedly different depending on p53 context. In tumors from p53+/+ Lck-Bax38/1 mice, hyperdiploidy

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<td>p53+/+Lck-Bax38/1</td>
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<tr>
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<td>p53−/−Lck-Bax38/1</td>
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<td>37, XY, –4, der(9)t(9;16), der(12)t(4;12), –16, –19 [3]/37, idem. –2, –18 [1]/38, idem. +16 [1]/38, idem. +19 [1]/76, XXY, –4, –4, der(12)t(4;12), der(12)t(4;12), –16 [1]</td>
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<tr>
<td>Four additional cells with 47–49 chromosomes unrelated to the above cells all sharing unbalanced translocations</td>
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<td>der(12)t(12;14) and der(14)t(14;14) in addition to multiple aberrations that make each cell unique from one another.</td>
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<td>48,XY, +1,+4, +5, +8, +10, +11, +14, +15/49, idem. +4/3]/50, idem. +4, +7[2]</td>
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<td>Seven nonclonal abnormal cells, related to the abnormal clones, with multiple numerical and structural aberrations.</td>
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was detected primarily with the gain of one or two chromosomes in each case. Of note, chromosome 15 was frequently gained in these tumors. In contrast, p53−/−Lck-Bax38/1 tumors were found to have more complex karyotypes with the gain or loss of multiple chromosomes and/or the presence of unbalanced translocations (Table 1). These results suggest that Bax expression promotes CIN and are consistent with other studies that show the absence of p53 markedly exacerbates this phenotype. Consistent with this interpretation and previous studies, we also observed complex karyotypes in thymic lymphomas from p53−/− mice (data not shown).

Bax-induced aneuploidy in 6- to 10-week-old premalignant Lck-Bax38/1 mice is attenuated by Bcl-2. If CIN drives tumor formation in this model, one would predict that aneuploidy precedes tumorigenesis in Lck-Bax38/1 mice. To test this prediction, quantitative cytogenetic analysis was done on thymocytes isolated from 6- to 10-week-old p53+/+ and p53−/− mice. In these experiments, cytogenetic samples were prepared directly from the mice without in vitro culturing to assure that the observed aneuploidy occurred in vivo. For each individual mouse, mitotic spreads were stained with Giemsa and photographed, and the number of chromosomes in each metaphase spread was quantified. The distribution of chromosome number from individual mice varied between the groups. The p53−/− control mice (Fig. 1A–C) had rare aneuploid cells that most commonly differed from diploid cells by the loss of one or two chromosomes. Because similar profiles were observed in p53+/+ mice (data not shown), this may represent infrequent technical artifacts from slide preparation. In p53−/− mice expressing Bax, the numerical karyotypes observed were highly variable. Several of these mice had numerically distinct aneuploid populations, in many cases gaining more than five chromosomes (Fig. 1D–F). The pattern of aneuploidy in p53+/+ Lck-Bax38/1 mice was intermediate between the controls and p53−/−Lck-Bax38/1 mice. Although the total percentage of aneuploid cells was markedly higher in p53+/+ Lck-Bax38/1 transgenic mice relative to controls, the aneuploid cells generally differed from diploid by the gain of one or two chromosomes (Fig. 1G–I). The detection of numerically distinct aneuploid populations in the p53−/−Lck-Bax38/1 mice (Fig. 1D and E) suggests that independent aneuploid populations may have emerged.

In Fig. 2A, the percentage of aneuploidy for each individual mouse in the study is shown. Based on a cutoff of 10%, enforced Bax expression in Lck-Bax38/1 mice significantly increased aneuploidy as compared with their transgene negative controls (Fig. 2A). In p53+/+ mice, this phenotype was not 100% penetrant at this age because three out of eight mice had levels of aneuploidy

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Increased aneuploidy in thymi from 6- to 10-week-old Lck-Bax38/1 mice. Representative histograms illustrating the pattern of thymic aneuploidy observed in 6- to 10-week-old transgenic mice are shown. Three different animals from p53−/− control mice (A–C), p53−/−Lck-Bax38/1 mice (D–F), and p53+/+Lck-Bax38/1 (G–I) are shown.
similar to transgene negative controls. In contrast, all six p53−/− mice expressing Bax were observed to be aneuploid. Of note, six out of seven of the p53−/− transgene negative controls were normal, with the other mouse being just above the cutoff (Fig. 2A). Bcl-2 expression was fully able to attenuate aneuploidy in Lck-Bax38/1 p53+/+ mice. Transgenic Bcl-2 expression alone did not induce aneuploidy in this age range (Fig. 2A). To show that these differences in ploidy are specific to thymocytes, lipopolysaccharide-stimulated B cells cultured in vitro from Lck-Bax38/1 transgenic mice did not have any significant differences in aneuploidy (data not shown). Importantly, the Lck-Bax38/1 mice had hypocellular thymus relative to control mice and showed that none of these animals had overt lymphomas (Fig. 2B).

Aneuploidy in 3- to 6-week-old premalignant Lck-Bax38/1 mice. To determine if aneuploidy is present in younger Lck-Bax38/1 transgenic mice, quantitative cytogenetic analysis and DNA content analysis were carried out on mice between 3 and 6 weeks of age. Propidium iodide staining of thymocytes isolated from p53−/−Lck-Bax38/1 mice frequently revealed the presence of more than one G0-G1 peak, consistent with an aneuploid population, whereas all p53+/+ mice expressing transgenic Bax were similar to controls (data not shown). Quantitative cytogenetic analysis revealed six out of eight p53−/−Lck-Bax38/1 mice had thymic aneuploidy levels above the 10% cutoff, which was significantly higher than the p53−/− controls (Fig. 2C). Four of these mice had very high levels of aneuploidy (>50%), which again seemed to represent multiple independent aneuploid populations (data not shown). p53−/− controls as well as p53+/+ mice with or without transgenic Bax expression did not show an increase in aneuploidy in this age range. As seen in the older cohort, all the Lck-Bax38/1 transgenic mice had markedly hypocellular thymus, excluding the presence of an overt lymphoma (Fig. 2D). Together, these results strongly suggest that CIN is an early event in premalignant p53−/−Lck-Bax38/1 transgenic mice.

SKY analysis of 3- to 6-week-old premalignant p53−/−Lck-Bax38/1 mice reveals oligoclonal aneuploidy. Because several of the p53−/−Lck-Bax38/1 transgenic mice showed aneuploid populations with distinct numbers of chromosomes (Fig. 1D and E). SKY analysis was done to determine if these abnormal populations share common cytogenetic aberrations. SKY analysis of three mice between 3 and 6 weeks of age was done. In the first mouse, three cytogenetically independent populations were observed. Two of these populations harbored unbalanced translocations: an aneuploid population with der(9)(9;11) and a pseudodiploid population with a der(9)(t(4;9)). Moreover, another pseudodiploid population had a unique chromosomal aberration involving a portion of chromosome 11 inserted into chromosome 1 and was trisomic for chromosome 16. Of note, three other cells shared the der(9)(t(4;9)) translocation but had additional cytogenetic aberrations (Fig. 3A). In the second mouse, three separate populations with unique unbalanced chromosomal translocations were identified. Two of these populations were pseudodiploid and had unbalanced translocations involving chromosome 12: one population harboring a der(12)(t(4;12)) and the other a der(12)(t(12;16)). The der(12)(t(12;16)) clone had a subpopulation that was trisomic for chromosome 15. A third clonal population contained an unbalanced translocation, der(9)(t(9;19)), and gained nine chromosomes (Fig. 3B). Of note, a der(12)(t(4;12)) was also detected in one of the tumors (Table I). However, these translocations seem distinct because the tumor involved a centromeric region of chromosome 4 (data not shown), whereas the translocation in the premalignant sample involved a more distal region of chromosome 4. In the third mouse, two cytogenetically independent populations had unique unbalanced translocations, der(12)(t(12;16)) and der(12)(t(Y;12)), respectively. A third independent population gained four additional chromosomes but had no translocations (Fig. 3C). SKY analysis of three p53−/− control mice in this same age range did not reveal any numerical or structural aberrations. A summation of all SKY analysis in 3- to
Figure 3. Oligoclonal aneuploidy and pseudodiploidy in 3- to 6-week-old p53–/–Lck-Bax38/1 mice. Thymocytes isolated from three (A–C) 3- to 6-week-old p53–/–Lck-Bax38/1 transgenic mice were subject to SKY analysis. *Top left corner,* histogram panel illustrating the pattern of aneuploidy observed for each mouse. *Red bar,* percentage of numerically diploid cells. Each subsequent panel within each montage illustrates individual cells observed from that mouse with the karyotypes listed above. The bracketed numbers following each karyotype indicates the number of cells bearing that karyotype. *Red boxes* within each SKY, chromosomal aberration(s) observed for that cell.
6-week-old mice is presented (Table 2). Furthermore, because the mitotic cells within the thymus are almost all of T-cell lineage, the aneuploid cells observed by SKY in this study are almost certainly of T-cell origin. These data show that oligoclonal aneuploidy is highly prevalent in pretumorigenic tissue in p53−/−/Lck-Bax38/1 mice.

Table 2. SKY analysis of 3- to 6-wk premalignant p53−/− thymi

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<th>Genotype</th>
<th>Karyotype</th>
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<td>p53−/−Lck-Bax38/1</td>
<td>39, XY,−19, der(12)t(4;12) [1]/40, XY,−16,+4, der(12)t(12;16) [5]/40, XY, der(12)t(4;12) [3]/49, XY,+1,+2,+4,5,+8,+14,+15,+18, der(X)t(X)19 [1]</td>
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<td></td>
<td>39, XY,−16, der(12)t(12;16) [4]/40, XY [2]/40, XY,−16,+1 fragment, der(12)t(12;16) [1]/41, XY, der(12)t(Y;12),+15 [3]/44, XY,+4,+5,+10,+15 [5]</td>
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<td>39, XY,−11, der(9)t(9;11) [2]/40, XY,−11,+16, ins(1;11) [2]/40, XX [1]/40, XX, der(9)t(4;9),+15,+15 [2]/44, XX, der(9)t(4;9),+14,+15,+15 [1]</td>
</tr>
<tr>
<td>p53−/−</td>
<td>40, XY [10]</td>
</tr>
<tr>
<td></td>
<td>40, XY [9]/39, XY,−2 [1]</td>
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<td>40, XY [12]</td>
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Centrosome amplification in premalignant Lck-Bax38/1 transgenic mice. Abnormal centrosome amplification is associated with and posited to be a potential mechanism that leads to CIN (23). To determine if centrosome amplification is associated with CIN in Lck-Bax38/1 transgenic mice, centrosome immunofluorescence was carried out on thymic tumors and premalignant thymocytes isolated from 3- to 6-week and 6- to 10-week-old mice. Centrosomes were stained with an anti-β-tubulin antibody, and the number of centrosomes per cell was enumerated. Supernumerary centrosomes (three or more centrosomes per cell) were detected in Lck-Bax38/1 thymic tumors (Fig. 4A). Because aneuploidy was an early event in p53−/−Lck-Bax38/1 mice between the ages of 3 and 6 weeks, it was hypothesized that centrosome aberrations would also be present in these mice. There was a significant increase in supernumerary centrosomes in p53−/−Lck-Bax38/1 transgenic mice as compared with their transgene negative controls (Fig. 4B). Interestingly, p53+/+Lck-Bax38/1 mice in this age window did not display increased supernumerary centrosomes relative to controls. In 6- to 10-week-old p53+/+Lck-Bax38/1 mice, there was a significant increase in supernumerary centrosomes as compared with transgene negative controls (Fig. 4C). Bcl-2 expression attenuated the increase in supernumerary centrosomes observed in Lck-Bax38/1 mice. Moreover, Bcl-2 expression alone did not affect supernumerary centrosome levels. These data provide evidence that supernumerary centrosomes are an early precursor lesion in pretumorigenic thymi in Lck-Bax38/1 transgenic mice.

Discussion

It is widely accepted that oncogenesis is a multistep process that occurs from the accumulation of alterations to the genome. These alterations may manifest as either gross chromosomal changes or subtle sequence changes. In either case, genetic instability is thought to be an enabling characteristic of oncogenesis (18). Despite this model, whether CIN is causative or simply a product of cancer remains controversial (24). Of note, patients with HNPCC have defects in mismatch repair that result in microsatellite instability, but the tumors are chromosomally stable (20). These data suggest that malignant transformation is not sufficient to cause CIN and suggest that increased rates of mutation are achieved via distinct and mutually exclusive mechanisms.

Of note, HNPCC is inherited in an autosomal dominant fashion with a defect in one of the genes involved in mismatch repair. Loss of the normal allele and development of MIN precede mutations in APC and suggest that the MIN phenotype occurs very early in oncogenesis (21). These results show that the development of MIN is an initiating event in HNPCC. By analogy, we predicted that CIN would also be an initiating event in cancer and, more specifically, that Lck-Bax38/1 transgenic mice would show high levels of aneuploidy (possibly oligoclonal) in mice at a point before overt tumor formation.

CIN is prevalent in human malignancy; however, the pathways leading to CIN during oncogenesis are poorly understood. p53 is a multifunctional transcription factor involved in many different cellular processes, including cell-cycle checkpoints, senescence, DNA damage response and repair, apoptosis, and maintenance of genomic integrity. Previous findings showed that in the absence of p53, fibroblasts grown in vitro rapidly become aneuploid (25) and mice deficient in this gene are predisposed to developing spontaneous aneuploid tumors (26, 27). In fact, one report found a high degree of aneuploidy in many otherwise normal tissues from p53−/− mice (28). In contrast to these findings, inactivation of p53 in HCT116 human colon cancer cells did not induce aneuploidy (29). Furthermore, other studies in colorectal cancer suggested that aneuploidy preceded p53 mutation (30), and that p53 gene mutation is a late event in tumorigenesis (31). Moreover, in a murine epithelial transgenic brain tumor model, inactivation of p53 (via selective pressure in p53+/− mice) did not result in CIN (32). Some of these discrepancies may be explained by differences in species, cell type, or other genetic/strain differences. However, the preponderance of data support a model in which p53 mutations may not be the primary cause of CIN, but instead exacerbates it (20). In concordance with this, our karyotype data in both malignant and premalignant settings clearly show striking differences in the aneuploidy observed in Lck-Bax38/1 mice depending on p53 context. In the 6- to 10-week-old p53+/+Lck-Bax38/1 mice, three of the mice had levels of aneuploidy similar to that observed in controls, suggesting that the phenotype may be age dependent. Consistent with this, aneuploidy was not detected in the p53+/+Lck-Bax38/1 mice between 3 and 6 weeks of age. However, in p53−/− mice, Bax expression markedly increased aneuploidy in both age groups. The increased aneuploidy observed in the p53−/− mice also correlated with a more rapid onset of tumor formation.
formation as previously shown (12). Of note, in the p53−/−Lck-Bax38/1 mice in the 3- to 6-week age group, two of the eight mice had aneuploidy below the 10% cutoff. These data again suggest that the phenotype is age dependent even in the absence of p53 and supports a model that loss of p53 exacerbates CIN, perhaps by decreased apoptosis of aneuploid cells (33, 34).

As mentioned previously, numerous reports have shown that the Bcl-2 family members have a paradoxical effect on tumor development and prognosis. In this study, we found that Bcl-2 blocked Bax-induced aneuploidy in p53+/+ mice. These effects could be explained by the retention of wild-type p53 in cells expressing Bcl-2 as has been observed in other models (17, 35–37). However, this simple model does not fully explain the paradoxical effects observed in our studies. Previous studies showed that Bax expression promotes tumor formation even in p53−/− mice, and tumor formation is delayed by the coexpression of Bcl-2 (12). In this study, Bax expression markedly increased aneuploidy in p53−/− mice relative to the p53−/− controls. Clearly, in this model, both Bax and Bcl-2 are able to exert paradoxical effects on oncogenesis even in p53−/− mice.

Recent studies have linked the BH3-only family member Bid to the DNA damage response (38, 39). These studies showed that Bid−/− cells were highly susceptible to DNA-damaging agents and showed that Bid is phosphorylated by ATM following DNA damage, and Bid is required for S phase arrest. Although the implications for these findings to the studies described here are not clear, one possibility is that excess Bax expression in our model binds to and inhibits this novel function of Bid. The resulting cells may then have an impaired DNA damage response that may increase CIN. Although this is an attractive hypothesis, it should be noted that another recent report failed to show these defects in Bid−/− mice (40).

Centrosomes are the principal microtubule-organizing centers (MTOC) in metazoans and are responsible for the proper segregation of chromosomes during mitosis (41). Many studies in both human and animals have documented centrosomal defects in tumors. Supernumerary centrosomes have been found in cancers of the breast, prostate, non-Hodgkin's lymphoma, brain, as well as many other cancers (23, 41). Supernumerary centrosomes have been proposed to cause CIN by promoting mitotic defects (multipolar mitoses) that, in turn, lead to aneuploidy. Centrosomal defects have been shown to be early events in tumor formation in animal models (42, 43) and coincided with CIN in cancers of the cervix, breast, and prostate (44). Despite these observations, the mechanistic significance of these defects in causing CIN is still subject to debate. Of note, ectopic expression of pericentrin

Figure 4. Supernumerary centrosomes in Lck-Bax38/1 transgenic mice. A, representative cells from a p53+/+Lck-Bax38/1 thymic tumor (top), a 6- to 10-week-old p53+/+ control thymus (middle), and a 6- to 10-week-old p53−/−Lck-Bax38/1 thymus (bottom) were stained for γ-tubulin (red), α-tubulin (green), and nuclei (blue) as described in Materials and Methods. The percentage of thymocytes with supernumerary centrosomes from 3- to 6-week-old (B) and 6- to 10-week-old (C) mice is illustrated. *, percentage of cells harboring supernumerary centrosomes from an individual mouse. Groups were compared using the Fisher's exact test with a cutoff of 2%. †, P < 0.05, versus transgene negative controls; ††, P < 0.01, versus transgene negative controls in the age groups, respectively. Bcl-2 significantly decreased the level of supernumerary centrosomes in 6- to 10-week-old Lck-Bax38/1 mice. ††, P < 0.05.
a major component of the centrosome) resulted in centrosomal defects, aneuploidy, and a tumor-like phenotype in prostate cancer cell lines (45). In concordance with previous findings of centrosomal defects in malignant tissue, tumors from Lck-Bax38/C0 transgenic mice displayed supernumerary centrosomes. Furthermore, we show that in premalignant thymi, Bax expression increased the incidence of cells with supernumerary centrosomes. As observed for aneuploidy, Bax expression increased supernumerary centrosomes in p53+/−/ mice at 6 to 10 weeks, but not in the 3- to 6-week age group. However, in p53−/−/ mice, Bax expression markedly increased the incidence of supernumerary centrosomes even in 3- to 6-week mice.

To our knowledge, this study is the first to use SKY analysis to show oligoclonal aneuploidy in otherwise normal murine tissue. A striking finding in the mice analyzed in this study revealed chromosomal aberrations in otherwise normal diploid populations as detected by quantitative cytogenetic analysis. Moreover, in all mice analyzed, cytogenetically distinct populations were detected as shown by the presence of unique translocations. Another feature in these mice is the detection of multiple subpopulations of cells that share a common translocation. This all occurs in the context of a very young animal with a markedly hypoplastic thymus. From these SKY snapshots, one senses the clonal selection and evolution that are occurring and driving oncogenesis in this primordial milieu. These studies support a novel link between the Bcl-2 family and CN and provide an alternative model for the paradoxical effects of the Bcl-2 family on oncogenesis.

Acknowledgments


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References

Correction: CIN in Bax-Induced Lymphoma

In the article on CIN in Bax-induced lymphoma in the September 1, 2007 issue of Cancer Research (1), Mary C. Horne (Program in Molecular Biology and Department of Pharmacology, University of Iowa, Iowa City, Iowa) should have been included as the second author.

Chromosomal Instability and Supernumerary Centrosomes Represent Precursor Defects in a Mouse Model of T-Cell Lymphoma

Christopher I. van de Wetering and C. Michael Knudson


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