Synergistic Role of Ku80 and Poly(ADP-ribose) Polymerase in Suppressing Chromosomal Aberrations and Liver Cancer Formation

Wei-Min Tong, Ulrich Cortes, M. Prakash Hande, Hiroko Ohgaki, Luciane R. Cavalli, Peter M. Lansdorp, Bassem R. Haddad, and Zhao-Qi Wang

ABSTRACT

Liver cancer is one of the major human tumors in the world. Basic and epidemiological studies have proposed that the major risk factors for liver cancer include alcohol and diet as well as infection with hepatitis B and C viruses. However, the mechanistic clues for the development of this type of cancer is largely unknown. Poly(ADP-ribose) polymerase (PARP-1) and a component of nonhomologous end-joining (NHEJ) machinery, Ku80, are two major DNA end-binding molecules that play a multifunctional role in DNA damage signaling and repair, recombination as well as the maintenance of genomic stability. Here we show that the interaction of PARP-1 and Ku80 is essential for development because PARP-1/Ku80 double null mice died at embryonic day (E) 9.5. Interestingly, haplo-insufficiency of Ku80 in PARP-1−/− mice promotes the development of hepatocellular adenoma and hepatocellular carcinoma (HCC). These tumors exhibited a multistage tumor progression associated with the loss of E-cadherin expression and the mutation of β-catenin. Cytogenetic analysis revealed that Ku80 heterozygosity elevated chromosomal instability in PARP-1−/− cells and that these liver tumors harbored a high degree of chromosomal aberrations including fragmentations, end-to-end fusions, and recurrent nonreciprocal translocations (NRT). These features are reminiscent of human HCC. Taken together, these data implicate a synergistic function of Ku80 and PARP-1 in minimized chromosome aberrations and cancer development and suggest that defects in DNA end-processing molecules may be etiological factors in human HCC formation.

INTRODUCTION

Genomic integrity and an intact DNA repair process are essential for normal development and for tumor suppression (1–3). PARP-1 is a nuclear enzyme that catalyzes the poly(ADP-ribosylation) of target proteins in response to DNA damage and has been proposed to play a role in DNA repair/recombination and cell death and proliferation, as well as in stabilization of the genome (4, 5). Although mice lacking PARP-1 develop normally (6–8), and exhibit normal DNA excision repair and unaltered V(D)J recombination as well as immunoglobulin class switching, PARP-1 null mice are hypersensitive to high doses of γ-irradiation (Refs. 7, 9, 10; see also Ref. 11). PARP-1 null cells exhibit an increased degree of chromosomal instability characterized by an increased frequency of spontaneous SCE and DNA damage-induced micronuclei formation (7, 9). Moreover, PARP-1 mutant mice and cells show shortened telomeres and chromosome aberrations, including chromosome fusions and fragmentation as well as chromosome loss and gain (12–14). These data suggest that PARP-1 plays an important role in stabilizing the genome, most likely via shutting on and off DNA strand breaks. Previous studies have shown that, although PARP-1−/− mice rarely develop spontaneous tumors (summarized in Ref. 11), in a p53-deficient background, these mice show increased tumor incidence and develop a wide spectrum of tumors including a high frequency of epithelial malignancy and brain tumors, in addition to sarcomas and lymphomas. This is most likely attributable to a combined effect of p53-mediated checkpoint failure, together with a high degree of chromosomal aberrations and impaired telomere function (14).

PARP-1 is proposed to be an anti-recombinogenic factor because it temporarily occupies DNA breaks to prevent accidental recombination between homologous DNA available in a local environment (4). Its inactivation, thus, results in a hyper-recombination activity as judged by spontaneously high levels of SCE (7, 9). Genetic studies, using PARP-1-deficient SCID mice in which DNA-PKcs is mutated, have further tested this elevated recombination activity. Although a large proportion (~75%) of PARP-1/SCID double mutant mice die soon after birth, PARP-1 deficiency partially rescues T-cell development in the survivors because of improved V(D)J recombination in these cells (15). However, these mice succumbed to T-cell lymphoma, most likely because of increased abnormal V(D)J recombination (15).

Ku80 is a DNA-binding subunit of the DNA-PK complex that is a major component of NHEJ machinery and is involved in DNA double-strand break repair and antigen receptor V(D)J recombination (16). Mice lacking Ku80 are growth retarded and show deficiency in V(D)J recombination (17, 18) and hypersensitivity to γ-irradiation. Ku80−/− cells exhibit significant DNA repair defects (19, 20), telomere shortening, and chromosome instability (21–23). Although Ku80−/− mice rarely develop spontaneous tumors (16), these mice in a p53-deficient background exhibit a high penetrance of pro-B-cell lymphoma caused by the specific translocation of c-myc and IgH locus (22, 24). Although the increased cytogenetic aberrations have been detected in Ku80 heterozygous mutant cells (23), its relevance to cancer has not been reported.

Given the high affinity of PARP-1 and Ku80 for DNA strand breaks and their colocalization at telomeres (4, 12, 16, 23), it is speculated that an interaction of these two molecules at DNA ends may be important for genomic stability and for other chromatin functions. Biochemical studies have shown that on DNA damage, PARP-1 and Ku80 are rapidly activated and compete in DNA end binding (25), and that a complex of PARP-1 and Ku proteins can be coinmunoprecipitated (26), suggesting that these two DNA-break-sensing molecules interact in response to DNA strand breaks in vitro. However, the role of this interaction in vivo is largely unknown.

In the present study, we investigated the biological significance of the interaction between PARP-1 and Ku80, and questioned whether the hyper-recombination induced by PARP-1 deficiency would facil-
Ku80 HETEROZYGOSITY PROMOTES HCC IN PARP-1−/− MICE

MATERIALS AND METHODS

Animals and Genotyping. PARP-1−/− mice (7) were maintained in a pure 129/Sv background for more than four generations before breeding to Ku80+/− mice (Ref. 17; 129/GyC57BL/6-3bino). To introduce a null PARP-1 allele into Ku80 knockout mice, PARP-1+/−/Ku80+/− mice were intercrossed. Genotyping for PARP-1 was carried out by PCR as described previously (14). Genotyping for Ku80 was carried out by PCR as described by Vogel et al. (27).

Histopathological Analyses. Animals were killed by ether anesthesia on decline in health (i.e., weight loss, paralysis, ruffling of fur, or inactivity). A full necropsy was performed and organs were fixed in 4% neutral-buffered formaldehyde, followed by dehydration and paraffin embedding. Histopathological analysis was carried out on 3-μm-thick section stained with H&E. Immunostaining was essentially as described previously (14). Antibodies were monoclonal antimouse E-cadherin (ECCD-2; Zymed Laboratories, San Francisco, CA) and monoclonal antimouse β-catenin (Clone 14; Transduction Laboratories, Lexington, KY).

Southern Blotting, SSCP Analysis, and Direct DNA Sequencing. For SSCP analysis, HCC samples from four PARP-1+/−/Ku80+/− and seven PARP-1+/−/Ku80+/− male mice at age 15–24 months were screened for mutations in exon 3 of the β-catenin gene (28). DNA was extracted from tumor cells microdissected from paraffin sections, and samples that showed mobility shift in SSCP analysis were further analyzed by direct DNA sequencing.

Cytogenetic Analyses and SKY. Primary MEFs were isolated from embryos derived from intercrosses of PARP-1+/−/Ku80+/−, or of PARP-1+/−/Ku80+/−, according to protocols described previously (7). For the fluorescence in situ hybridization (FISH), metaphase spreads were prepared following standard procedures and were stained with DAPI. Telomere staining was performed using Cy-3-labeled (CCCTAA)3 peptide nucleic acid (14). SKY of the primary HCC cells was performed as described previously (29). For the SKY hybridization, whole-chromosome mouse probes were used (Applied Spectral Imaging, Inc.). At least 10 metaphases were analyzed for each of the HCC cases. Structural aberrations were determined to be clonal if found in two or more metaphases.

RESULTS

Null Mutation of Both PARP-1 and Ku80 Results in Lethality during Mid-Gestation. Although PARP-1−/−/Ku80+/− and PARP-1+/−/Ku80−/− mice were born from intercrosses of PARP-1+/−/Ku80+/− mice, after analyzing 746 newborns, we did not observe double null offspring (PARP-1−/−/Ku80−/−), which suggests that the homozygous disruption of both the PARP-1 and Ku80 genes causes embryonic lethality. To define embryonic stages of gestation at which double null embryos die, embryos at various stages, derived from intercrosses of PARP-1+/−/Ku80+/− or of PARP-1+/−/Ku80+/− mice were genotyped by PCR analysis. At embryonic day (E) 13.5, among 71 embryos genotyped, no PARP-1−/−/Ku80−/− fetuses were found (Fig. 1A). However, 8 PARP-1+/−/Ku80−/− embryos were readily identified at E9.5 after 44 embryos were analyzed (Fig. 1A). These fetuses were distinguishable from their littermates by being smaller in size, having 15–18 somites (versus 28–32 in normal embryos), and not having turned red completely (Fig. 1B), indicative of growth retardation. To further define the stage when the growth retardation starts, we genotyped 74 embryos at E8.5 and 63 at E7.5. Whereas no obvious morphology alterations were found with E7.5 embryos, the smaller size of PARP-1−/−/Ku80−/− embryos could be detected at E8.5 (Fig. 1A). Together, PARP-1−/−/Ku80−/− embryos became growth retarded as early as E8.5 and died shortly after E9.5.

PARP-1 and Ku80 Synergistically Minimize Chromosomal Instability. To investigate the impact of PARP-1 and Ku80 on chromosomal integrity, we performed cytogenetic analysis on chromosomal structures from MEFs derived from PARP-1+/−/Ku80+/− intercrossing. Whereas 10% of PARP-1+/−/Ku80+/− MEFs were aneuploid and these cells contained various chromosome aberrations, 33% of PARP-1+/−/Ku80−/− mutant cells were aneuploid and showed more complex cytogenetic changes (Table 1). Strikingly, 52% of metaphases that were derived from PARP-1+/−/Ku80+/− MEFs displayed numerous chromosomal aberrations, including Robertsonian translocation, dicentrics, and chromosomal fragments/breaks compared with those in PARP-1+/−/Ku80+/+ or PARP-1+/−/Ku80−/− cells (Table 1), indicating that Ku80 heterozygosity enhanced chromosomal abnormalities of PARP-1−/− cells. Furthermore, PARP-1+/−/Ku80−/+ MEFs exhibited intermediate increase of end-to-end fusions (27%) and fragmentation (21%; Table 1). These data suggest that PARP-1 and Ku80 play a synergistic role in minimizing the aberrant joining of chromosome ends as well as chromosomal fragmentations.

PARP-1/Ku80-deficient Mice Develop a High Frequency of Liver Tumors. PARP-1 deficiency-induced telomere dysfunction and chromosomal instability facilitate tumor development in a p53 mutant background (14) and Ku80 null mutant cells suffer severe genomic instability and telomere dysfunction (21, 23). Interestingly, Ku80+/− cells exhibited a strong haplo-insufficiency phenotype in terms of the effect of Ku80 knockout in telomere length as well as genomic stability (23). To test whether Ku80 heterozygosity might affect survival and tumor development in PARP-1-deficient mice, a large cohort of mice of various genotypes was monitored for a period of 12–24 months. Systematic macroscopic and microscopic analyses revealed a high frequency of tumor development in PARP-1/Ku80 double mutant mice (Table 2). Specifically, 40 (63%) of 64 PARP-
Primary MEs (the number of passages, <3) of given genotypes were used to prepare metaphases. Chromosome numbers and aberrations were scored in 16–102 metaphase spreads of each genotype after DAPI and FISH staining.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Metaphase analyzed</th>
<th>Aneuploidy (%)</th>
<th>End-to-end fusions (%)</th>
<th>Fragments/ breaks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP-1/+/+ Ku80+/+</td>
<td>102</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (1.9) 1AC 1C</td>
</tr>
<tr>
<td>PARP-1/+/- Ku80+/+</td>
<td>16</td>
<td>2 (12.5)</td>
<td>2 (12.5)</td>
<td>2AC</td>
</tr>
<tr>
<td>PARP-1/-/- Ku80+/+</td>
<td>49</td>
<td>5 (10.2)</td>
<td>12 (24.5)</td>
<td>6AC 2C 4Br</td>
</tr>
<tr>
<td>PARP-1/+/- Ku80+/-</td>
<td>46</td>
<td>15 (32.6)</td>
<td>21 (45.6)</td>
<td>15AC 2C 4Br</td>
</tr>
<tr>
<td>PARP-1/+/- Ku80+/-</td>
<td>16</td>
<td>2 (12.5)</td>
<td>2 (12.5)</td>
<td>2AC</td>
</tr>
<tr>
<td>PARP-1/+/- Ku80+/+</td>
<td>48</td>
<td>4 (8.3)</td>
<td>10 (20.8)</td>
<td>7AC 3Br</td>
</tr>
<tr>
<td>PARP-1/-/- Ku80+/-</td>
<td>31</td>
<td>4 (12.9)</td>
<td>16 (51.6)</td>
<td>7AC 2C 7Br</td>
</tr>
</tbody>
</table>

a The percentage in the parentheses represents events per 100 cells and breakdown of chromosomal abnormalities.

1/+-Ku80+/- and 72 (79%) of 91 PARP-1/-/- Ku80+/- mice developed tumors after 24 months of age with mainly epithelial tumors, whereas 6 (10%) of 58 control mice (wild-type, PARP-1/+/- Ku80+/+ and PARP-1/+/- Ku80++/+ developed tumors in 24 months (Table 2). Notably, frequent hepatocellular adenomas and carcinomas (HCC) were observed in aged (12–24 months) PARP-1/-/- Ku80+/+ (28%) and PARP-1/-/- Ku80+/+ mice (60%; Table 2; Fig. 2, A and B). These liver tumors were predominantly associated with male PARP-1-deficient Ku80+/-/- mice (75% of liver tumor-bearing mice). Histological analysis revealed that numerous foci of adenoma were presented in various lobes of liver (Fig. 2C); and in those, HCC tumor cells were arranged often as trabecular or pseudoglandular structures (Fig. 2D), characteristic of the multistage progression. It was also noted that 10 (28%) of 36 PARP-1/-/- Ku80++/+ mice developed liver tumor, i.e., 7 with hepatocellular adenomas and 3 with HCC (Table 2).

Loss of E-Cadherin Expression and Mutation of β-Catenin are Associated with Development of Hepatocellular Adenoma and Carcinoma. To further characterize the tumor progression, we examined the status of E-cadherin and β-catenin at the different stages of these liver tumors in PARP-1/-/- Ku80+/+/- mice. Immunohistological analysis showed that E-cadherin was expressed at low levels at the cell membrane of normal hepatocytes but increased in hepatocellular adenomas (Fig. 2E). Strikingly, E-cadherin expression was largely undetectable in HCC (Fig. 2F), reminiscent of human HCC. Moreover, the progression changes of E-cadherin expression were evident in a subset of liver tumors containing mixed areas of adenoma and carcinoma, in which expression of E-cadherin was focally lost only in the HCC areas (data not shown). These data are consistent with the notion that down-regulation or loss of E-cadherin is involved in the transition from adenoma to carcinoma (30).

We next analyzed expression of β-catenin in these mice and found no difference in β-catenin expression pattern between normal hepatocytes and adenoma cells, namely localization on the hepatocyte membrane (Fig. 2G). During the progression of adenoma to HCC, β-catenin expression accumulated in the cytoplasm, and then in the nucleus (Fig. 2H). This transition of expression pattern was evident in 55% of the tumors analyzed. The nuclear accumulation of β-catenin correlated well with the proliferation of tumor cells as evidenced by positive staining for proliferating cell nuclear antigen (PCNA) (data not shown). The alteration of E-cadherin and β-catenin expression has also been seen in the tumor sections derived from PARP-1/-/- Ku80+/+ and PARP-1/-/- Ku80+/-/- mice (data not shown).

Because the relocation of β-catenin to cell nuclei in tumors is often caused by mutations in this molecule (31, 32), mutation of the β-catenin gene was analyzed by SSCP and subsequent sequencing. Microdissection of tumor areas from histological sections was performed and tumor DNA was subject to PCR amplification. SSCP analysis revealed band shifts of β-catenin in 4 of the 11 HCC tumors analyzed (data not shown). Subsequent sequencing of PCR products from these tumor cells showed four GAC→GCC mutations in codon 17 and one ACC→GCC mutation in codon 40 of the β-catenin gene (Fig. 2I), indicating frequent β-catenin mutations associated with HCC development in PARP-1-deficient Ku80+/-/- mice.

High Rate of Chromosomal Abnormalities in HCCs Derived from PARP-1/-/- Ku80+/+/- Mice. To understand the genetic basis of enhanced tumor development, we performed cytogenetic analysis on chromosomal structures from primary HCC cells derived from PARP-1/-/- Ku80+/+/- mice. SKY analysis of metaphases prepared from primary HCC cells revealed both structural and numerical changes of chromosomes in tumor cells (Fig. 3). In tumor N1014, 42% of the cells analyzed were near-tetraploid, whereas the rest of the cells were near-diploid (40–42 chromosomes); all of the cells analyzed in tumor N1029 had a near-diploid karyotype (40–43 chromosomes). In N1029 tumor cells, we detected a consistent NRT between chromosomes 11 and 19 in every cell, t(11;19) (Fig. 3, A and B), suggesting clonal chromosomal aberrations. We confirmed the t(11;

Table 1 Impact of PARP-1 and Ku80 deficiency on chromosomal integrity

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Metaphase analyzed</th>
<th>Aneuploidy (%)</th>
<th>End-to-end fusions (%)</th>
<th>Fragments/ breaks (%)</th>
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<td>2 (1.9) 1AC 1C</td>
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<tr>
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<td>46</td>
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<tr>
<td>PARP-1/+/- Ku80+/-</td>
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<tr>
<td>PARP-1/-/- Ku80+/-</td>
<td>31</td>
<td>4 (12.9)</td>
<td>16 (51.6)</td>
<td>7AC 2C 7Br</td>
</tr>
</tbody>
</table>

a The percentage in the parentheses represents events per 100 cells and breakdown of chromosomal abnormalities.

Table 2 Spectrum of spontaneous tumors a

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Control b (n = 18)</th>
<th>PARP-1/-/- Ku80+/+ (n = 10)</th>
<th>PARP-1/-/- Ku80+/+ (n = 54)</th>
<th>PARP-1/-/- Ku80+/- (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular adenoma</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>HCC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemangiosarcoma in liver</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other epithelial tumors b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total tumors</td>
<td>0</td>
<td>6</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

a Mice at the age of 12–18 months and 18–24 months (mo) were grouped for monitoring and histological examination. The number in parentheses corresponds to the number of mice in each group.

b Control group included mice with genotypes of wild-type, PARP-1/+/- Ku80+/+, and PARP-1/+/- Ku80+/-/.

c Including tumors in the lung, skin, and prostate.
19) finding using standard FISH analysis with chromosomes 11- and 19-specific painting probes (Fig. 3C). On the basis of the appearance of this chromosome in an inverted DAPI image and the FISH and SKY results, this derivative chromosome [t(11;19)] is consistent with a pseudodicentric chromosome composed of the proximal regions of chromosomes 11 and 19 (Fig. 3B). In addition, all of the metaphases had a Robertsonian translocation either involving the two chromosome 1 homologues, rob(1;1) (Fig. 3, A and B) or between chromo-

### Table: Mouse Heterozygosity Promotes HCC in PARP-1−/− Mice

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Age (months)</th>
<th>Sex</th>
<th>β-catenin mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N514</td>
<td>15</td>
<td>M</td>
<td>codon 17, GAC → GCC, Asp → Ala</td>
</tr>
<tr>
<td>N783</td>
<td>20</td>
<td>M</td>
<td>codon 17, GAC → GCC, Asp → Ala</td>
</tr>
<tr>
<td>N853</td>
<td>24</td>
<td>M</td>
<td>codon 17, GAC → GCC, Asp → Ala</td>
</tr>
<tr>
<td>N882</td>
<td>22</td>
<td>M</td>
<td>codon 40, ACC → GCC, Thr → Ala</td>
</tr>
</tbody>
</table>

Fig. 2. Histological and genetic analyses of liver tumors in PARP-1−/− Ku80 +/− mice. Macroscopic view of a normal liver (A), and a massive liver tumor (B). Bar, 1 cm. C, a hepatocellular adenoma. D, a HCC exhibiting a distinct trabecular pattern with some areas of glandular structures. E-H, immunohistochemical staining of adjacent sections from hepatocellular adenoma (a; E and G) or carcinoma (F and H) with antibodies against E-cadherin (E and F) or β-catenin (G and H). There is a lack of E-cadherin expression in the nontumoral part of the liver (n). Focal loss of E-cadherin in the carcinoma (F, left) is shown. β-cadherin expression is primarily on the cell membrane of normal hepatocytes (not shown) and in hepatocellular adenoma (G) and in nuclei in carcinoma (H). I, sequencing analysis of tumor DNA showing specific mutations in the β-catenin gene in HCC tumors. C-H, ×40.
somes 1 and 2, rob(1;2), (Fig. 3, D and E). Small acentromeric markers (fragmentations) originating from different chromosomes including 9 and 14 were detected in 33% of the metaphases (Fig. 3, D and E). In tumor N1104, we detected a Robertsonian translocation involving chromosomes 2 and 18, rob(2;18), in over 90% of the cells with near-diploid complement and in about 10% of the near-tetraploid cells (Fig. 3, D and E). These cytogenetic data indicated both clonal and nonclonal NRTs in these HCCs.

DISCUSSION

Although PARP-1 and Ku80 mutant mice are viable, PARP-1/Ku80 double null mutation causes early embryonic lethality, suggesting a synergistic role between PARP-1 and Ku80 in developmentally essential processes, most likely in DNA damage-mediated cellular responses such as DNA damage signaling and repair. The absence of both molecules leads to the accumulation of unrepaired chromosome breaks, which renders the embryonic cells susceptible to apoptosis. In this regard, it is interesting to note that PARP-1 null mutation in mice lacking ataxia telangiectasia mutated (ATM) causes early embryonic lethality most likely caused by increased apoptosis (33). In addition, it is also possible that the accumulation of chromosome damage in cells deficient in both PARP-1 and DNA-PK pathways may compromise cell proliferation. This hypothesis is supported by the observation that PARP-1+/−/Ku80−/− mice exhibited severe growth retardation compared with PARP-1+/+Ku80−/− mice, and the majority of these mice died shortly after birth or before weaning.4 The survivors at weaning were 40% of the bodyweight of their wild-type, and 65% the bodyweight of their PARP-1−/−/Ku80−/− littermates, throughout their life, reminiscent of small body sizes of PARP-1−/−/SCID mice (15). These observations indicate that functional synergy between DNA damage-signaling molecules is essential for embryonic development.

Although PARP-1 mutant mice did not show development of spontaneous tumors in previous studies (6–8), PARP-1 null mice showed an enhanced tumor susceptibility in the SCID background (15). In addition, it has recently been shown that there is an increase in the formation of sarcomas and adenomas when PARP-1 mutant mice are treated with nitrosamine (34). PARP-1−/−/Ku80−/+ mice in the present study exhibited a relatively high frequency (28%) of liver lesions, suggesting that after sequential intercrossing of PARP-1−/−/mice, presumably having eliminated the potential interference by genetic background, the colony becomes predisposed to liver

4 W-M. Tong and Z-Q. Wang, unpublished observations.

<table>
<thead>
<tr>
<th>Tumor samples</th>
<th>No. of metaphase</th>
<th>Ploidy level</th>
<th>% of cells</th>
<th>No. of cells</th>
<th>% of chromosomal translocations and structural aberrations</th>
</tr>
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<tbody>
<tr>
<td>N1104</td>
<td>50</td>
<td>near-tetraploid</td>
<td>42</td>
<td>20</td>
<td>-t(2;18): 90% in near-diploid cells, 10% in near-tetraploid cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>near-diploid</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1029</td>
<td>50</td>
<td>near-diploid</td>
<td>100</td>
<td>13</td>
<td>-t(11;19): 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-Robertsonian translocations:</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85% rob(1;1) and 15% rob(1;2)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>-Acentromeric markers: 33%</td>
</tr>
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</table>

Fig. 3. SKY analysis of primary HCC cells. In A, chromosome spreads were prepared from HCC cells (N1029 and N1104) derived from PARP−/−/Ku80+/− mice. A representative metaphase showing the NRT between chromosomes 11 and 19 [t(11;19)] and the Robertsonian translocation involving the two chromosome 1 homologues, [rob(1;1)]. B, the translocation chromosomes in the metaphase shown in A, visualized in display colors (middle row), inverted DAPI (left row), and after spectra-based classification in pseudocolors (right row). C, confirmation of the chromosomal origin of the t(11;19) using chromosomes 11- (green) and 19- (red) specific painting probes. D, chromosomal aberrations detected in other metaphases from tumors N1104 [rob(2;18)], and N1029 [rob(1;2)], and a small acentromeric marker originating from chromosome 14. E, summary of chromosomal translocations and aberrations in primary HCC cells, N1029 and N1104. Ploidy of the tumor cells was determined by scoring at least 50 chromosome spreads.
malignancy. Although we cannot formally rule out the possibility that a modifier locus linked to the PARP-1 knockout allele contributes to HCC development, several lines of evidence support the hypothesis that PARP-1 may be a limiting factor for hepatocyte transformation. For example, the susceptibility of PARP-1 null mice to liver lesions has also been observed in a 129/Sv background after multiple intercrosses of the PARP-1−/− genotype. In addition, the inhibition of PARP-1 activity by 3-aminobenzamide (3-AB) enhances liver carcinogenesis in rats induced by diethylnitrosamine (35) or phenobarbital (36). This notion is also supported by the observation that male rats fed with a niacin-deficient diet, resulting in low PARP-1 activity, are susceptible to HCC (37). Our preliminary data have also shown that PARP-1−/− mice are susceptible to diethylnitrosamine-induced liver tumors. Finally, because the liver is the major detoxification organ that is constantly exposed to genotoxic agents and metabolites, it is plausible that a proficient DNA repair machinery would be critical to prevent accumulation of genetic mutations in tumor suppressors and oncogenes.

Strikingly, PARP-1−/− mice, carrying a heterozygous mutation of Ku80, exhibit enhanced HCC formation as reflected by the 60% incidence in PARP-1−/−/Ku80+/− mice compared with 28% in PARP-1−/−/Ku80+/+ genotype. This is apparently consistent with the observation that haplo-insufficiency of another NHEJ molecule Lig4 induces soft tissue sarcomas in Ink4a/arf−/− mice (38). These findings are in agreement with the notion that the compromised NHEJ activity induces tumor development in murine models as well as in humans (reviewed in 39, 40). Although mutations of components of the NHEJ pathway cause lymphoid malignancy (Ku80 in Ref. 22, 24; DNA-PKcs in Ref. 15, 41, 42; Lig4 in Ref. 43; and XRCC4 in Ref. 44) and soft tissue sarcomas (Lig4 in Ref. 38), Ku80 heterozygosity promotes the transformation of hepatocytes. All of these data point to NHEJ activity as possibly playing an important role in suppressing the malignancy of various cell origins.

One of the hallmarks of malignant transformation is genomic instability caused by mutations in genome caretaker genes, which promotes a wide range of genetic mutations, chromosome deletion and translocations, gene amplification, and aneuploidy (1–3). Because PARP-1 and Ku80 function in DNA repair and recombination and telomere regulation as well as in chromosome stabilization (9, 12, 13, 21–23), both molecules can synergistically maintain telomere function (see Ref. 14) and minimize aberrant chromosome end-joining, and thereby, suppress tumorigenesis. This hypothesis is supported by the observation that an increased frequency of chromosome end-to-end fusions and fragmentations/breaks was found in PARP-1−/−/Ku80−/− MEFs, when compared with that observed in PARP-1−/−/Ku80−/+; PARP-1+/+Ku80−/− and PARP-1+/−/Ku80−+/− cells (see Table 1). Moreover, our SKY analysis has shown that primary HCC cells derived from PARP-1/Ku80 double mutant mice exhibit frequent end-to-end fusions, NRTs, and Robertsonian-like configurations, as well as a high degree of fragmentation. A plausible explanation for these cytogenetic alterations is that the persistence and aberrant repair of double-strand breaks caused by reduced Ku80 activity and PARP-1 mutation provide substrates for the end-joining of chromosomes, the quality of which is otherwise monitored by the NHEJ machinery (2). On the other hand, because PARP-1 is proposed to be an anti-recombinogenic factor (4) and PARP-1−/− cells exhibit a high degree of SCE (7, 9), this elevated recombinogenic activity induced by PARP-1 deficiency may facilitate aberrant joining of chromosome ends stalled by Ku80 deficiency leading to chromosomal translocations and fusions.

Although the functional alterations and phenotypic consequences of genes at break/fusion points remain to be investigated, we were able to show a high frequency of E-cadherin and β-catenin alterations in HCCs of these double mutant mice. The activation of oncogenes or loss of inhibitory effect mediated by adhesion molecules has been proposed in neoplastic transformation and tumor progression (30). In this regard, we found frequent down-regulation or loss of E-cadherin expression in these HCCs (see Fig. 2). These data are consistent with the notion that down-regulation or loss of E-cadherin is involved in tumor progression in animal models and human samples (30). Moreover, specific gene mutations, such as those in β-catenin, render hepatocytes susceptible to a selective growth advantage leading to cancer (32).

These HCCs, prevalent in males and exhibiting a typical multistage progression characterized by the gradual loss of E-cadherin and the nuclear accumulation of β-catenin, as well as chromosome translocations and fragmentations, are reminiscent of human HCC (31, 32, 45–50). Cytogenetic studies of human HCC have revealed frequent abnormalities in chromosome 1q, such as translocation, trisomy, or amplification (45, 47, 51) and, also, loss of heterozygosity of chromosome 1q42–43 and 2q35–37 in human HCC (52, 53). These studies imply that HCC-susceptible genes may localize at chromosome 1q and/or 2q. It is interesting to note that PARP-1 and Ku80 are located at human chromosome 1q41–42 (54) and 2q33–34 (55), respectively. However, further investigation is required on whether the imbalance or loss of chromosome 1q may alter human PARP-1 gene expression or its ADP-ribosylation activity, and whether loss of heterozygosity of 2q35–37 in humans may lead to Ku80 deficiency, together with PARP-1 dysfunction, contributing to HCC formation.

HCC is one of the most common human malignant tumors in the world. Although infections by hepatitis B and C viruses, excessive alcohol intake, and exposure to aflatoxin B1 are major etiological factors for liver cancer, the molecular changes leading to hepatocyte transformation and cancer development remain largely unknown, partly because of the limited animal models available. The present study has demonstrated the interaction of PARP-1 and Ku80 as genome caretakers in suppressing liver tumorigenesis and the role that the NHEJ pathway plays in suppressing tumorigenesis. The high incidence of liver tumors (60%) in PARP-1-deficient Ku80−/+ mice is associated with the molecular and genetic alterations that often occur in human HCC; therefore, these mice represent a potential model for human HCC, which would allow the exploration of the molecular and genetic mechanism of HCC and of the possible applications in developing therapeutic strategies.

ACKNOWLEDGMENTS

We thank Drs. David Roth, Baylor College of Medicine, Houston, Texas, and Paul Hasty, Lexicon Genetics Inc., The Woodlands, Texas, for providing Ku80 knockout mice. We are also grateful to Dominique Galedo, Christelle Granier, Nicole Lyandrat, and Anne-Marie Camus-Randon for the maintenance of the animal colonies and technical assistance. Further thanks are due to Dr. Lucien Frappart for histopathological examination and Dr. Cristina Montagna, Bethesda, Maryland, for providing the chromosome-specific probes. We also thank Drs. Alain Barbin, Zdenko Herceg, and Ciaran Morrison for their critical comments, and Eve El-Akroud for editing the manuscript.

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