Loss of \textit{Rad51c} Leads to Embryonic Lethality and Modulation of \textit{Trp53}-Dependent Tumorigenesis in Mice

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Abstract

RecA/Rad51 protein family members (Rad51, Rad51b, Rad51c, Rad51d, Xrcc2, and Xrcc3) are essential for DNA repair by homologous recombination, and their role in cancers has been anticipated. Here we provide the first direct evidence for a tumor suppressor function for a member of the Rad51 family. We show that \textit{Rad51c} deficiency leads to early embryonic lethality, which can be delayed on a \textit{Trp53-null} background. To uncover the role of \textit{Rad51c} in tumorigenesis, we have exploited the fact that \textit{Rad51c} and \textit{Trp53} are both closely located on the mouse chromosome 11. We have generated double heterozygous (\textit{DH}) mice carrying mutant alleles of both genes either on different (\textit{DH-trans}) or on the same chromosome (\textit{DH-cis}), the latter allowing for a deletion of wild-type alleles of both genes by loss of heterozygosity. \textit{DH-trans} mice, in contrast to \textit{DH-cis}, developed tumors with latency and spectrum similar to \textit{Trp53} heterozygous mice. Strikingly, \textit{Rad51c} mutation in \textit{DH-cis} mice promoted the development of tumors of specialized sebaceous glands and suppressed tumors characteristic of \textit{Trp53} mutation. In addition, \textit{DH-cis} females developed tumors significantly earlier than any other group. [Cancer Res 2009;69(3):863–72]

Introduction

DNA repair protects the genome from acquiring mutations that may potentially lead to cellular transformation and tumorigenesis. DNA double-strand breaks, the most severe type of DNA lesions, are repaired either by nonhomologous end joining or by homologous recombination pathways (1). In homologous recombination, genetic information from a homologous region of a sister chromatid is used as a template to faithfully restore the damaged DNA. Rad51 is a key protein in the homologous recombination pathway, mediating strand invasion and exchange between a free DNA end proximal to a damaged site and a homologous double-stranded DNA (2). Mammalian cells possess an elaborate molecular machinery to ensure a timely and precise loading of Rad51 at sites of DNA damage. Rad51 is a key protein in the homologous recombination pathway, mediating strand invasion and exchange between a free DNA end proximal to a damaged site and a homologous double-stranded DNA (2). Mammalian cells possess an elaborate molecular machinery to ensure a timely and precise loading of Rad51 at sites of DNA damage. This machinery includes five members of the RecA/Rad51 family (Rad51b, Rad51c, Rad51d, Xrcc2, and Xrcc3) that show 20% to 30% sequence identity to Rad51 (3). These Rad51 paralogues interact with each other and can be purified as two protein complexes (4, 5). One of these complexes includes Rad51b, Rad51c, Rad51d, and Xrcc2 (BCDX2 complex), and the other contains Rad51c and Xrcc3 (CX3 complex). Functional analysis of DNA repair proficiency of double mutants in chicken DT40 cells suggests that these two protein complexes have distinct functions because Xrcc3 and Rad51d double-mutant cells display an additive effect on sensitivity to cisplatin compared with single mutants (6). Each paralogue within the BCDX2 complex also seems to contribute differently to the common function based on differential sensitivity to DNA-damaging agents. Rad51c is part of both of these complexes and is thought to play a central role in these associations. Functional analysis has shown that all these genes specifically affect the homologous recombination pathway and suppress recruitment of Rad51 to the site of DNA damage (6, 7). Cell lines lacking any of these genes are sensitive to DNA cross-linking agents and are genomically unstable and accumulate chromosomal rearrangements (6, 8). In addition to these five paralogues, another member of this family, DMC1, shows 50% sequence identity to Rad51. It is a structural and functional homologue of Rad51 that functions specifically in meiotic recombination (9).

Although a link between \textit{Rad51} family members and cancer is expected, results implicating these genes in cancer thus far have been circumstantial (see ref. 10 for review and references). Overexpression of a dominant-negative form of Rad51 in Chinese hamster ovary cells increased tumorigenesis when these cells were transplanted into nude mice. Down-regulation of Rad51 was found in patients with multiple myeloma. The product of a balanced translocation between RAD51B (RAD51L1) and a high mobility group protein, HMGA2, with subsequent loss of the second RAD51B allele, has been implicated in uterine leiomyomas. RAD51D was found to play an important role in telomere maintenance and its E233G variant may be a low-penetrance allele in high-risk breast cancer families without mutations in BRCA1 and BRCA2. A marginal increase in risk ratio has also been found for several XRCC2 and XRCC3 sequence variants in connection with breast and some other types of cancer. RAD51C (RAD51L2) is part of a 17q23 cytoband frequently amplified in sporadic breast cancers (10, 11).

Although the above-mentioned studies show association of Rad51 family members with cancer, additional verification with larger number of patients is needed (12). In addition, animal studies implicating \textit{Rad51} and its paralogues in cancer have been hampered due to early embryonic lethality of mice null for most of these genes (13–18). We have previously reported generation of a conditional allele of \textit{Rad51c} in mouse, where exons 2 and 3 were floxed by insertion of a floxed \textit{PGK-neo} cassette in intron 1 and a single \textit{loxP} site in intron 3 of the \textit{Rad51c} gene (19, 20). We used these mice to show the role of Rad51c in meiotic recombination. Here we describe generation of a null allele of \textit{Rad51c} in mice. We show that \textit{Rad51c} is essential for mouse embryonic development and provide evidence that \textit{Rad51c} functions as a tumor suppressor. This study will set a paradigm for the role of other \textit{Rad51} family members in tumorigenesis.
Materials and Methods

Construction of the targeting vector. The KpnI-EcoRI fragment (13.354 kb) of the genomic region containing the first four exons of Rad51c was subcloned from the BAC clone RPCI22-514-2C into pBSK(+) plasmid to produce pCSL5 construct. Thymidine kinase (TK) gene (NotI-BamHI fragment) under the control of the MC1 promoter was subcloned into pBSK (+) (pSKS11), and subsequently, its NotI-Asp718 fragment was inserted into pCSL5 to produce pCSL5TK construct. EcoRV-BstXI fragment (2 kb) from pLMJ237 plasmid containing loxP-PGK-Trs5-neomycin-bp(A)-loxP was inserted into intron 3 of Rad51c in pCSL5TK construct using the lambda-Red recombineering approach (21) and the selection cassette was then deleted by Cre-mediated recombination in bacteria leaving behind a 103-bp-long insert containing a single loxP site (pCSL5TKA). In addition, an EcoRV-BstXI fragment (2 kb) from pLM260 containing an frt-loxP-PGK-EM7-neomycin-bp(A)-frt-loxP cassette was targeted into the first intron of Rad51c using lambda-Red recombineering system (pCSL5TKAB8). The resultant targeting vector had a 6.7-kb homology arm upstream of the first loxP site and a 3.8-kb homology arm downstream of the last loxP site.

Targeting of Rad51c in embryonic stem cells and generation of mutant mice. Targeting vector was linearized with Sall and electroporated into CJ7 embryonic stem cells derived from 129S1/SvImJ mouse line. Electroporation and selection were done with the CJ7 embryonic stem cell line as described elsewhere (22). 127 G418 R, FIAUR embryonic stem cell lines were subcloned from the BAC clone RPCI22-514-2C into pBSK(+) plasmid for 40 cycles at 94°C for 40 s, 53°C for 40 s, and 72°C for 40 s. The PCR product was subcloned into the BAC clone RPCI22-514-2C into pBSK(+) plasmid to produce pCSL5 construct. 

The entire Rad51c gene was amplified from pCSL5TK plasmid by PCR using primers F: 5'-TGCCTGAATGTGTCTGCAC-3' and R: 5'-ATAGCAGGCAGCAGCATCT-3' (for the restriction site map). One of these embryonic stem cell clones was correctly targeted. The presence of the last loxP site was confirmed by hybridizing SpeI-digested genomic DNA with an internal probe (see Fig. 1A for the restriction site map). One of these embryonic stem cell clones was injected into C57BL/6 blastocysts to generate chimeras. One of these chimeras transmitted the targeted allele, Rad51cneo/+, denoted as Rad51cneo/+, in the germ line, and Rad51cneo/+ pups were obtained. To produce a null allele, Rad51cneo/+, denoted as Rad51cneo/+, Rad51cneo/+- mice were crossed to β-actin-Cre-deleter strain (23). Rad51cneo/+- mice have a mixed genetic background inherited from C57BL/6 and 129S1/SvImJ mouse strains, with C3H and CD1 backgrounds from 129S1/SvImJ and CD1 strains. The colony was maintained on a mixed genetic background. Littermate controls were used in all studies. Mice were maintained under limited-access conditions at the National Cancer Institute (Frederick) and animal care was provided according to the procedures outlined in the Guide for the Care and Use of Laboratory Animals under an approved animal care and use committee protocol.

Genotyping of Rad51c and Trp53 mutant mice. For genotyping purposes, genomic DNA was extracted from tail biopsies or from frozen tumor tissues according to standard procedures. To genotype for Rad51c by Southern blot, 3 to 5 μg of tail DNA were digested overnight with Hpal, size-fractionated in a 0.8% agarose gel in Tris-acetate-EDTA buffer for 6 h to resolve 10- and 12-kb bands, and transferred onto a N+-Hybond nylon membrane (Amersham) by the alkaline transfer method. Rad51c internal probe (Int2), 531 bp long, was amplified from pCSL5TK plasmid (F: 5’-TGCTGAATGTGTCTGCAC-3’; R: 5’-ATAGCAGGCAGCAGCATCT-3’) for 40 cycles at 94°C for 40 s, 57°C for 40 s, and 72°C for 40 s. The PCR product was subcloned into the BAC clone RPCI22-514-2C into pBSK(+) plasmid to produce pCSL5 construct. 

Figure 1. Mouse Rad51c gene targeting. A, scheme illustrating the gene targeting strategy to generate a Rad51c-null allele. Rad51c exons are indicated as boxes with corresponding numbers. Restriction sites: S, Sall; H, Hpal; R, EcoRI; K, KpnI. F1, F2, and R1 designate location and direction of primers used for PCR-genotyping. Hpal restriction fragments detected by Southern blot analysis with the internal probe b are indicated as blue lines under each allele. A single loxP site was inserted at the third intron (at genomic location chr11: 87217150) and a single loxP site was inserted at the third intron (at genomic location chr11: 87214383). B, depiction of exon structure of the Rad51c transcript and the corresponding protein with functionally important regions. NH2-terminal domain is shown in green and COOH-terminal domain is shown in blue. C, genotyping by Southern blot showing four different genotypes with allele sizes labeled on the right. D, examples of genotyping using PCR primers shown in A.
product was then gel-purified and labeled with \[^{32}P\]dCTP using the Random Primer Labeling Kit according to the manufacturer’s instructions. Hybridization was conducted according to standard procedures and band sizes corresponding to Rad51c alleles were interpreted as shown in Fig. 1C. Genotyping for Trp53 by Southern blotting was done as described elsewhere (24).

We also designed a PCR genotyping strategy to genotype Rad51c and Trp53 mutant mice. For Rad51c, ~50 ng of tail or embryo DNA were amplified with the Platinum Taq polymerase (Invitrogen) using a three-primer strategy (F1: 5′-ACCAGGCGGTGGTGGCCAGCGCTTTAATCTCCGACTTG-3′; F2: 5′-CAATGCTGGAATATAAGACCCTGTGCTACATCCAAAGTG-3′; R1: 5′-GCGTATCATACATCACAGCTGATCTCAGGGAG-3′) at 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s for 25 cycles (after initial 2 min at 94°C). PCR products were analyzed by electrophoresis on a 1% agarose gel. The size of PCR products was 414 bp for a null (ko) allele, 524 bp for the wild-type, and 627 bp for the conditional (25) allele (Fig. 1D).

For Trp53, tail DNA was amplified with the Platinum Taq polymerase (Invitrogen) using a three-primer strategy [F1: 5′-CCTCAATAAGC-GAGGCTCAGGTGACAGCTT-3′; F2: 5′-CGTGAATGCTGGAAGAGGCTCAGGT-3′; R2: 5′-CTGTCTGTCCAGATCGCTGACG-3′] at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, for 35 cycles (after initial 2 min at 94°C). PCR products were analyzed by electrophoresis on a 1% agarose gel. The size of PCR products was 370 bp for a mutant (ko) allele and 320 bp for the wild-type allele.

**Generation and aging of mouse cohorts.** We studied five mouse cohorts as described below. We used Trp53 heterozygous mice carrying Trp53^tm^/^bd^ allele, denoted as Trp53^ko^ (23). To produce the wild-type, Rad51c^eo/eo^ and Trp53^ko/ko^, as well as double-heterozygous animals with mutant alleles located on different homologous chromosomes (DH-trans), we intercrossed Rad51c^eo/eo^ and Trp53^ko/ko^ mice. Rad51c and Trp53 reside on mouse chromosome 11 only 10 cM apart and are therefore linked. To produce mice with mutant alleles on the same homologous chromosome (DH-cis), we first mated DH-trans mice with the wild-type and selected rare double-heterozygous progeny, which could be obtained only if the two mutant alleles recombined during meiosis. Such mice carrying the two mutant alleles on the same homologous chromosome were then backcrossed to the wild-type to increase the cohort size. Mice were group-housed with food and water ad libitum and were maintained on a 12 h light/12 h dark cycle. Animals were monitored for 600 d. Sick mice and those with visible tumors were sacrificed and sent for pathologic evaluation. One half of each tumor mass identify at necropsy was snap-frozen and stored at −80°C for further loss of heterozygosity (LOH) analysis.

**Immunostaining for MSH2.** To test whether a mismatch repair protein Msh2 was specifically lost in preputial and Zymbal’s gland tumors, we stained those tissues immunohistochemically. Paraffin slides were first deparaffinized with xylene and then rehydrated through four changes of 100% ethanol and one change of 95% ethanol. Endogenous peroxidase was blocked for 15 min in 0.6% H2O2 in methanol. Heat-induced epitope retrieval with 0.01 mol/L citrate buffer (BioGenex Laboratories) was carried out at 100°C for 10 min. The slides were then rinsed with PBS for 10 min, blocked with goat serum (Vector Labs) for 30 min, and incubated with primary anti-MSH2 antibody [Abcam; dilution 1:50 in PBS with 0.1% bovine serum albumin (BSA)] overnight at 4°C. Slides were then rinsed with PBS and incubated with the biotinylated secondary goat anti-rabbit antibody (Vector Labs) for 30 min, diluted 1:100 in PBS with 10% goat serum, followed by an avidin-biotin peroxidase complex method using the Vector Elite ABC kit (Vector Labs). Reaction was detected using 3,3′-diaminobenzidine and counterstained with hematoxylin and mounted in Permount (Dagger).

**Generation of Rad51c^−/−^ mouse embryonic fibroblasts in vitro and proliferation test.** Due to an early postimplantation lethality of Rad51c^−/−^ embryos, no Rad51c^−/−^ mouse embryonic fibroblast cell lines (MEF) could be established by a standard method. Therefore, we generated such cells by infecting MEFs that were homozygous for the conditional Rad51c allele (Rad51c^eo/eo^) with adenovirus expressing Cre-recombinase (AD-Cre-GFP, Viral Technology Laboratory, National Cancer Institute-Frederick). Primary MEFs were isolated from E13.5 F1 embryos obtained from a Rad51c^eo/eo^ mother backcrossed on C57BL/6 genetic background for seven generations and a Rad51c^eo/ko^ father backcrossed on 129/SvEv genetic background for eight generations. Exponentially growing MEFs (P1) were trypsinized and resuspended in a small volume of the culture medium (DMEM supplemented with penicillin and streptomycin and 15% fetal bovine serum) at 3 × 10^5^ cells/mL. A 100-μL aliquot containing 3 × 10^5^ cells was mixed with 3 μL of AD-Cre-GFP suspension (3 × 10^5^ viral particles) to achieve a multiplicity of infection ratio of 10. The mixture was allowed to stand for 1 h at room temperature and then split between two 10-cm tissue culture dishes. Infection efficiency was evaluated the next day by green fluorescent protein (GFP) expression. Genotyping for Rad51c by Southern blot was used to confirm 100% recombination efficiency. Four days after infection, the cells were collected and seeded at 0.3 × 10^6^ per 6-cm dish in duplicates for a proliferation assay according to the T33 protocol. Cells were collected and counted every 3 d and seeded again at 0.3 × 10^6^ per dish for six consecutive passages. Average increase in cell number was calculated for each passage and plotted as a cumulative growth.

**Isolation of Rad51c^−/−^ null MEFs from embryos.** To isolate Rad51c^−/−^ null MEFs, on Trp53^−/−^ null background, we intercrossed a pair of DH-cis mice and dissected embryos at E10.5. At that point, double-mutant embryos appeared morphologically similar to normal E9.5 embryos. Cells were explanted from embryo carcases into 10-cm tissue culture dishes by standard techniques and incubated at 3% oxygen concentration to suppress cellular senescence (26). Initially, mutant cells grew poorly and had to be split at a very low dilution ratio for the first five to seven passages. At approximately P10, the cell lines stabilized and grew at a similar rate as control MEFs (data not shown). Control Trp53^+/-^ MEFs were isolated from E9.5 embryos essentially the same way.

**Drugsensitivity test.** We tested the effect of DNA-damaging compounds on mutant cell proliferation in vitro. Two independent cell lines were tested for each genotype: Rad51c^−/−^; Trp53^−/−^ double-null, Trp53^−/−^ single-null control, and the wild type. We seeded 4,000 cells per well in gelatinized 24-well plates in duplicates for each line. Continuous drug treatment was started in 18 h at the following doses: mitomycin C (MMC) at 0, 10, 20, and 30 ng/mL and melythel sulfate at 0, 5, 10, and 15 μg/mL. Cells from one plate were trypsinized and counted using a Coulter counter and used as a control for plating efficiency and as a “before treatment” day 1 reference. Two days later (3rd day after seeding), the remaining cells were counted the same way. Day 1 reference numbers were subtracted from day 3 cell numbers to evaluate growth of each cell line. The resulting cell counts were expressed as percentages from the untreated wells.

**Rad51c and formation assay.** We plated equal numbers of cells per well in gelatinized Tissue Culture Treated Glass slides (Falcon). We irradiated slides 48 h later with 10 Gy. Six hours after irradiation, the cells were fixed with 4% paraformaldehyde for 5 min, washed twice with PBS, and permeabilized in PBS-buffered 0.1% Triton X-100 for 10 min. After two additional washes with PBS, we blocked cells in a blocking solution (1% BSA, 0.05% Triton X-100, 10% donkey serum in PBS). We performed antibody staining and imaging as described previously (27). Ten to 15 images with a total of at least 50 cells have been scored for Rad51 and γH2AX foci.

**Chromosomal aberration test.** We treated MEFs with colcemid (Invitrogen) for 1.5 h to arrest them at metaphase. The cells were then trypsinized, washed, and resuspended in hypotonic solution at 37°C (0.075 mol/L KCl) for 15 min and fixed in a methanol-acetic acid mixture (3:1, v/v). We stained air-dried preparations in Giemsa solution (10% Sorensen’s buffer and 2% Giemsa; J.T. Baker). Two hundred well-spread metaphases containing at least 40 chromosomes from each genotype were examined blindly for structural aberrations.

**Statistical analysis.** Animal survival and tumor latency data were processed using the survival/reliability function and P values were estimated using the Wilcoxon test using the JMP 5.0.1a statistical software package. Numbers of Rad51 and γH2AX foci in MEFs were evaluated using basic statistics functions in JMP. Because γH2AX foci showed a bimodal distribution, we separated the cells into groups A and B depending on the number of observed foci and evaluated them separately. Deviation of genotype segregation from the Mendelian ratio at various developmental
stages was calculated using $\chi^2$ test function in MS Excel (Supplementary Table S1).

Results

Gene targeting and embryonic lethality of Rad51cko/ko mice.

To generate a constitutive knockout allele, we crossed the mice carrying the floxed allele of Rad51c (Rad51cnosk, denoted hereafter as Rad51cneo) that retains the neomycin resistance gene (25) to mice expressing Cre recombinase under the control of the human $\beta$-actin promoter (28). The resulting allele, Rad51ctm1sks, denoted hereafter as Rad51cneo, lacked exons 2 and 3 that code for a 142-amino-acid region, including a linker region and an ATPase motif called Walker A (Fig. 1A and B). Walker A motif is indispensable for the function of Rad51 family proteins (19, 29). No mature Rad51c protein could be detected from this allele as has been shown previously (19, 29). We obtained Rad51c heterozygous (Rad51c+/-) mice that were viable and fertile and indistinguishable from their wild-type littermates.

When Rad51c+/- mice were intercrossed, we did not obtain homozygous mutant (Rad51c-/-) offspring (Supplementary Table S1), which suggested that these mice die during embryogenesis. In addition, the number of Rad51c+/- newborn mice relative to the wild-type (239 and 156, respectively) deviated from the Mendelian 2:1 ratio and indicated that some Rad51c+/- mice die during gestation. To determine the cause and time of lethality, we dissected embryos from a Rad51cko/ko intercross at various gestational stages between E5.5 and E10.5. At all stages with the exception of E8.5, ~25% of all embryos had an abnormal phenotype (Fig. 2; Supplementary Table S1). Genotyping of E7.5 and E8.5 embryos confirmed that phenotypically abnormal embryos were indeed Rad51cko/ko. At E8.5, 39% of all embryos (n = 126) were phenotypically abnormal. We found that 2 of 18 phenotypically abnormal embryos at this stage were heterozygous (Rad51c+/-) mice that were viable and fertile and indistinguishable from their wild-type littermates.

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for Rad51c, whereas the remaining 16 Rad51c<sup>ko/+</sup> embryos were indistinguishable from the wild-type littermates (Supplementary Table S1). This partial embryonic lethality of heterozygous mice may account for a sub-Mendelian ratio of Rad51c<sup>ko/−</sup> relative to wild-type newborn mice. Although its exact cause is currently unknown, we speculate that this may be due to mixed genetic background (see Discussion).

At E5.5, the mutant embryos lacked the proamniotic cavity and exhibited a slight delay in development (Fig. 2A, top). At E6.5 to E7.5, the delay in development of the embryo proper further increased whereas extraembryonic tissues continued to grow comparable to control littermates [Fig. 2A (bottom) and B (top)]. The degeneration processes became evident after E8.5 (Fig. 2B, bottom). At this stage, Rad51cko/ko embryos usually formed neural folds but looked severely abnormal and underwent resorption shortly thereafter. Histologic evaluation of the Rad51cko/ko embryos did not reveal failure of any specific tissue type, but rather an overall growth defect of embryonic tissues. Bromodeoxyuridine (BrdUrd) labeling of proliferating cells and terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining of cells with fragmented genomic DNA indicated that a marked increase in apoptosis occurred in the Rad51cko/ko embryos at the E8.5 developmental stage (Fig. 2C).

**Genetic interaction between Rad51c and Trp53.** Defects in DNA repair often lead to apoptosis, which can be ameliorated on a Trp53-deficient genetic background (14, 17). To examine whether the lethality of Rad51c-null embryos can be rescued by the lack of Trp53 and to have a better understanding of the cause of embryonic lethality, we crossed Rad51c<sup>ko/+</sup> mice to Trp53 heterozygous mice carrying Trp53<sup>tm1Brd</sup> allele, denoted hereafter as Trp53<sup>ko</sup> (24). We generated mice in which mutant alleles of these two genes (Rad51c<sup>ko/+</sup>;Trp53<sup>ko/−</sup>) resided in the same chromosome (double heterozygous-cis, DH-cis, Fig. 3A) and, thus, were inherited as a single genetic locus. No double-homozygous mutant (Rad51c<sup>ko/ko</sup>;Trp53<sup>ko/ko</sup>) offspring was obtained from DH-cis intercrosses. However, at E10.5, 25% of the embryos were confirmed to be Rad51c<sup>ko/ko</sup>;Trp53<sup>ko/ko</sup>. These mutant embryos are developmentally similar to an E10.5 embryo with a normal looking heart but the embryos are smaller in size (Fig. 2D). In addition, these embryos have truncated caudal region and unclosed head folds. The fact that Rad51c<sup>ko/ko</sup> embryos were severely degenerated after E8.5, and double-null embryos apparently progressed until E10.5 stage [Fig. 2B (bottom) and Fig. 2D], implied that embryonic development of Rad51c<sup>ko/ko</sup> mice could be partially rescued by the loss of Trp53. In addition, cells from Rad51c<sup>ko/ko</sup> embryos failed to proliferate in vitro but MEF lines from Rad51c<sup>ko/ko</sup>;Trp53<sup>ko/ko</sup> could be successfully generated (data not shown). These results suggest that the lethality of Rad51c<sup>ko/ko</sup> embryos is due to the apoptotic response to a DNA repair defect.

Rad51c has a gender-specific effect on tumor latency. To date, there is no definitive evidence that RAD51 or any of its paralogues functions as a tumor suppressor (12). It is likely that loss of any of these essential proteins triggers a severe proliferation defect, cell cycle arrest, and/or apoptosis (30). To examine the role
of Rad51c in tumorigenesis, we aged Rad51c<sup>−/−</sup> and wild-type littermates. In addition, to provide a cellular environment that lacks normal cell cycle checkpoints and allows proliferation of Rad51c<sup>−/−</sup> cells, we also monitored the tumor predisposition of Rad51c<sup>−/−</sup> mice on a Trp53<sup>+/−</sup> genetic background. We monitored the DH-cis mice (Fig. 3A), in which we expected a single LOH event to result in a simultaneous loss of the wild-type alleles of both Rad51c and Trp53. As a control, we used mice in which the mutant alleles of the two genes are on different homologues (double heterozygous-trans, DH-trans, Fig. 3A). We also aged Trp53<sup>−/−</sup> as a second control group to examine the effect of Trp53 loss alone.

We found no significant difference between the tumor latency of Rad51c<sup>−/−</sup> and wild-type control littermates, suggesting that heterozygosity for Rad51c alone does not increase the tumor susceptibility in mice (Table 1). However, among females, DH-cis mice developed tumors with a significantly shorter latency (369 days) than any other group including mice developed tumors with a significantly shorter latency susceptibility in mice (Table 1). However, among females, DH-trans and from other groups with a mutation in males did not show a significant difference in tumor-free survival of other groups with a mutation in Trp53. Interestingly, DH-trans and Trp53<sup>−/−</sup> males developed tumors earlier than females of the same genotype, whereas the correlation was reversed for DH-cis mice. We conclude that, first, the tumor latency of DH-trans is similar to that of Trp53<sup>−/−</sup> mice, and second, it is functionally important whether mutations of Rad51c and Trp53 are located on the same or on different homologous chromosomes.

**Tumor spectrum of DH-cis mice is different from those of DH-trans and Trp53<sup>−/−</sup> mice.** In addition to tumor latency, we also found significant differences in tumor spectrum between mice of different genotypes and genders. Consistent with previous reports (31, 32), the most common types of neoplasms in Trp53<sup>−/−</sup> females were osteosarcomas, mammary adenocarcinomas, and lymphomas (Table 2; Supplementary Table S2). Trp53<sup>−/−</sup> males, on the other hand, usually succumbed to muscle sarcomas, lung cancer, and hematopoietic malignancies. As with the tumor latency, the tumor spectrum of DH-trans mice was also remarkably similar to Trp53<sup>−/−</sup> mice. In contrast, DH-cis mice developed fewer tumors characteristic for Trp53-deficient mice, but revealed a greatly increased incidence of unique tumor types such as tumors of specialized sebaceous glands and tumors in the muzzle area (nasal and periorcular region, Table 2).

Preputial gland carcinoma was the most frequent type of sebaceous tumors in DH-cis males found in 10 of 24 (42%) animals. Four of these 10 animals additionally developed Zymbal's gland carcinomas and a fifth had a Harderian gland adenoma, all being glands secreting lipids. Another animal had a Zymbal's gland carcinoma only. We also found preputial gland carcinoma in one DH-trans male and a Zymbal's gland carcinoma in two DH-trans females. In all the mice we monitored, there was only one case of Zymbal's gland carcinoma in a mouse that did not have a Rad51c mutation (in Trp53<sup>−/−</sup> group).

Analysis of the tumor spectrum can also explain the differences in the tumor-free survival time between genders and different genotypes. Those neoplasms that seemed to be the probable cause of death or were a primary indication for clinical sacrifice in DH-cis females included mammary gland carcinomas and carcinomas of the skin and nasal malignancies, and were the major contributors to their shorter survival (average tumor latencies of 371, 323, and 331 days, respectively). A shift from aggressive Trp53-characteristic tumor types to preputial and Zymbal's gland carcinomas and hematopoietic neoplasms in DH-cis males (463, 446, and 398 days, respectively), was probably responsible for a longer survival in this group. In Trp53<sup>−/−</sup> and DH-trans males, muscle sarcomas were a single tumor type that had a decisive effect on the survival time (average latency was 308 days for 11 muscle sarcomas in Trp53<sup>−/−</sup> males and 255 days for 13 muscle sarcomas in DH-trans males).

**LOH in tumors.** In tumor cells, loss of the wild-type allele at a heterozygous locus for a tumor suppressor gene, like Trp53, is considered to be one of the primary mechanisms of tumor progression (33, 34). If LOH was indeed the leading mechanism of tumor initiation in this study, the tumor tissues from DH-trans mice would be genotypically identical to tumors from Trp53 heterozygous mice. In contrast, DH-cis mice should reveal the loss of both Rad51c and Trp53 genes. To test this, we genotyped the tumor samples collected from these animals by Southern blot analysis (Fig. 3D). As expected, all 24 tumor samples from Trp53<sup>−/−</sup>

### Table 1. Tumor-free survival varies with sex and genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender</th>
<th>Total no. of animals</th>
<th>Animals with tumors</th>
<th>Median tumor-free survival time (d)</th>
<th>P* (M vs F)</th>
<th>Differences between groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>F</td>
<td>17</td>
<td>2</td>
<td>&gt;600</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Rad51c&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>F</td>
<td>38</td>
<td>7</td>
<td>&gt;600</td>
<td>n.a.</td>
<td>vs WT P = 0.4402</td>
</tr>
<tr>
<td>Trp53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>F</td>
<td>11</td>
<td>8</td>
<td>475</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>DH-trans</td>
<td>F</td>
<td>26</td>
<td>21</td>
<td>461</td>
<td>n.a.</td>
<td>vs Trp53&lt;sup&gt;−/−&lt;/sup&gt; P = 0.8635</td>
</tr>
<tr>
<td>DH-cis</td>
<td>F</td>
<td>23</td>
<td>22</td>
<td>360</td>
<td>n.a.</td>
<td>vs DH-trans P = 0.0011</td>
</tr>
<tr>
<td>WT</td>
<td>M</td>
<td>21</td>
<td>3</td>
<td>&gt;600</td>
<td>0.7839</td>
<td>n.a.</td>
</tr>
<tr>
<td>Rad51c&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>M</td>
<td>32</td>
<td>6</td>
<td>&gt;600</td>
<td>0.9765</td>
<td>vs WT P = 0.5499</td>
</tr>
<tr>
<td>Trp53&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>M</td>
<td>20</td>
<td>16</td>
<td>385</td>
<td>0.2345</td>
<td>n.a.</td>
</tr>
<tr>
<td>DH-trans</td>
<td>M</td>
<td>24</td>
<td>22</td>
<td>334</td>
<td>0.0013</td>
<td>vs Trp53&lt;sup&gt;−/−&lt;/sup&gt; P = 0.4652</td>
</tr>
<tr>
<td>DH-cis</td>
<td>M</td>
<td>24</td>
<td>22</td>
<td>437</td>
<td>0.0466</td>
<td>vs DH-trans P = 0.0621</td>
</tr>
</tbody>
</table>

NOTE: P values were calculated using the Wilcoxon text.
Abbreviation: WT, wild type.
*Statistically significant differences are highlighted in bold.
mice showed the loss of the wild-type allele. Similarly, with the exception of one sample, all tumors from DH-trans mice (n = 33) lost the wild-type copy of Trp53 and the mutant allele of Rad51c. Of 32 tumors from DH-cis mice, 29 revealed the loss of the wild-type allele for both Trp53 and Rad51c. One DH-cis tumor sample lost a wild-type copy of Trp53 without losing Rad51c (Fig. 3D). The remaining two tumor samples did not reveal LOH for any of the two genes. Tumors from Rad51c<sup>neo/neo</sup> animals (n = 19) did not reveal LOH for Rad51c, except in one case. To test the possibility that Rad51c might be silenced epigenetically in these tumors, we examined its expression by reverse transcription-PCR. We found Rad51c expression by reverse transcription-PCR. We found Rad51c expression in all tumors tested (data not shown). Linking agent MMC or DNA alkylating compound methylmethane sulfonate. We found the Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> MEFs to be two to three times more sensitive to both agents compared with Rad51c<sup>ko/ko</sup> or wild-type MEFs (Fig. 4B). This observation is consistent with previously published data on Rad51c-deficient hamster CL-V4B and chicken DT40 cell lines (6, 36), although the degree of drug sensitivity of the MEFs was lower than those of hamster and chicken counterparts.

**Rad51c and sebaceous tumors.** As described above, DH-cis males were highly prone to tumors of specialized sebaceous glands, particularly preputial and Zymbal’s glands (Supplementary Fig. S1A and B). Sebaceous tumors from human patients often have a dysfunctional mismatch DNA repair pathway and are usually marked by down-regulation of Msh2 protein expression and microsatellite instability (35). Therefore, we tested 12 sebaceous tumors for five microsatellite markers (D1Mit62, D15Mit93, D17Mit72, urokinase-type plasminogen activator receptor, and pro-1) and found no evidence of microsatellite instability (data not shown). We also tested three of these tumors for expression of Msh2 on paraffin sections. In all three cases, the bulk of the tumor tissues stained strongly positive for this marker (Supplementary Fig. S2B), which may indicate either an up-regulation of this protein in the tumors or a neoplastic transformation of already existing Msh2-positive cells normally residing at the base of each follicle (Supplementary Fig. S2B). From these results, we concluded that a defect in mismatch repair was not responsible for sebaceous tumors in DH-cis mice.

**In vitro phenotype of Rad51c-deficient cells explains its function as a tumor suppressor.** How does loss of Rad51c promote tumorigenesis? To answer this question, we investigated the effect of Rad51c loss at the cellular level. Because of the early embryonic lethality, isolation of MEFs from Rad51c<sup>ko/ko</sup> embryos was not possible. Therefore, to generate Rad51c<sup>ko/ko</sup> MEFs, we infected Rad51c<sup>flo/flo</sup> MEFs expressing a conditional allele of Rad51c with adenovirus expressing Cre recombinase. The resultant Rad51c<sup>ko/ko</sup> MEFs, however, suffered a severe growth arrest after two to three passages (Fig. 4A), supporting the conclusion that Rad51c is essential for cell proliferation.

As reported previously (19), we obtained two independent Rad51c-null MEF lines from E10.5 Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> embryos (data not shown). We tested the ability of Rad51c-deficient cells to repair the DNA damage by challenging them with DNA-cross-linking agent MMC or DNA alkylating compound methylmethane sulfonate. We found the Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> MEFs to be two to three times more sensitive to both agents compared with Rad51c<sup>ko/ko</sup> or wild-type MEFs (Fig. 4B). This observation is consistent with previously published data on Rad51c-deficient hamster CL-V4B and chicken DT40 cell lines (6, 36), although the degree of drug sensitivity of the MEFs was lower than those of hamster and chicken counterparts.

Rad51c-deficient mammalian cells have been previously reported to have attenuated Rad51 foci after ionizing irradiation, thus implicating Rad51c in recruitment of Rad51 to sites of DNA damage and repair (6, 7). Whereas we observed an average of 26 foci in 62% of wild-type cells (n = 62) and 28 foci in 86% of Trp53<sup>ko/ko</sup> MEFs (n = 55) 6 hours after 10 Gy of γ-irradiation, no Rad51 foci could be found in Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> MEFs (Fig. 4C, Supplementary Table S3). In addition, unirradiated Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> MEFs revealed more γH2AX foci-positive cells (78% versus 22–39% in controls) than the control MEFs, suggesting the presence of abnormally large amount of damaged DNA even without exposure to any DNA-damaging agent.

Increased DNA damage and dysfunctional DNA repair lead to genomic instability. We determined the frequency of various chromosomal aberrations in mutant and control MEFs with or without treatment with a low dose of MMC (10 ng/mL) corresponding to a LD<sub>10</sub> for wild-type MEFs (Fig. 4B and C). Whereas an average of 106 chromosomal aberrations per 100 cells was observed in untreated Trp53<sup>ko/ko</sup> MEFs, their number increased to 150 in Rad51c<sup>ko/ko</sup>, Trp53<sup>ko/ko</sup> MEFs, suggesting that there is a constant level of DNA damage persisting in double-mutant cells even without a genotoxic treatment. This is consistent with the increased proportion of γH2AX-positive cells in unirradiated culture as described above. After treatment, the
number of chromosomal aberrations in Rad51cko/ko; Trp53ko/ko MEFs increased to 496 per 100 cells compared with only 170 aberrations per 100 cells in Trp53ko/ko MEFs. The most frequent types of aberrations were radial structures, chromosomal fragments, and chromatid gaps and breaks, which is consistent with the effect of MMC. It is worth noting that the number of chromosomal aberrations in primary wild-type and Rad51cko/+ MEFs was significantly lower compared with Rad51cko/ko; Trp53ko/ko MEFS. This may reflect disruption of some cellular pathways involved in chromosomal stability during establishment of the cell lines in culture. Taken together, tumors arising from DH-cis mice, which became functionally null for both Rad51c and Trp53 in most cases, are predicted to be deficient in DNA repair and genetically unstable and, thus, different from DH-trans tumors. This may have had a significant influence on the tissue specificity of tumors that developed in these mice as it is known for many other DNA repair genes.

Discussion

Functional analysis of Rad51 paralogues. Here we describe generation of a null allele of Rad51c and show that Rad51c is essential for viability in mice. Like Rad51c, loss of other Rad51 paralogues in mice also results in embryonic lethality. Rad51b-null embryos almost completely disappear as early as E7.5 and have the most severe phenotype among all paralogues (17). Rad51d-deficient embryos die between E9.0 and E10.0 (16). Xrcc2-mutant embryos develop normally through E8.5 (15). However, ~75% of the mutant embryos die between E10.5 and E12.5. Some even survive to birth but die within 20 minutes due to underinflated lungs. The phenotype associated with loss of Xrcc3 in mice is not known. Interestingly, the severity of the embryonic phenotype of Rad51b, Rad51c, and Rad51d directly correlates with respective cellular phenotypes in DT40 cells in terms of sensitivity to stalled replication forks induced by a topoisomerase I inhibitor, camptothecin, rather than relative sensitivity to DNA interstrand cross-links induced by cisplatin (6). In spite of their overlapping functions, each paralogue results in a distinct phenotype. This suggests that these paralogues may have other unique functions. Indeed, Rad51d is shown to be essential for telomere stability (37). A role in meiotic recombination and resolution of Holliday junctions has been shown for Rad51c and Xrcc3, and this function is evolutionary conserved through Arabidopsis thaliana (19, 38–40).

Rad51c is a tumor suppressor. Rad51c is evidently essential for DNA repair. Therefore, we expected an increase in tumor predisposition in Rad51cko/+ mice due to genomic instability in cells undergoing LOH at Rad51c locus. Failure to observe LOH in this group can be attributed to the fact that loss of the Rad51c function may be too detrimental for a cell, thus causing its elimination before the neoplastic transformation. The fact that during the 600-day period that we monitored the mice for tumors, the tumor latency, spectrum, and frequency did not significantly
differ between Rad51c<sup>ko/ko</sup> and wild-type mice supports our conclusion that heterozygosity for Rad51c alone does not predispose mice to cancer.

Because loss of Trp53 function can delay the onset of apoptosis in cells experiencing a severe proliferation defect due to Rad51c deficiency, we examined the tumor susceptibility of Rad51c<sup>ko/ko</sup> mice on a Trp53<sup>ko/ko</sup> genetic background. We monitored two classes (DH-trans and DH-cis) of genotypically identical (Rad51c<sup>ko/ko</sup>; Trp53<sup>ko/ko</sup>) mice. It was intriguing to find that these two classes produced distinctly different tumor types. LOH analysis revealed that most of the tumors from DH-cis mice lost the wild-type alleles of both Trp53 and Rad51c, whereas DH-trans mice lost the wild-type allele of Trp53 along with the mutant allele of Rad51c but retained its wild-type allele. This result strongly suggests that Rad51c plays an important role in tumor formation, albeit Trp53 dependent. Such dependence on Trp53 loss has been reported for many other tumor suppressor genes (e.g., BrcA1, BrcA2, Fbxw7/Cdc4; refs. 41–44). Although tumor latency in DH-cis mice is primarily determined by the LOH at the Trp53 locus, additional loss of Rad51c essentially overrides the tissue-specific effect of Trp53 mutation and promotes a shift from sarcomas (mesodermal origin) to malignancies of skin and adnexa (epidermal origin), especially those of specialized sebaceous glands and other glandular and neuroepithelial tissues such as those in the nasal and pericircular area. Therefore, we conclude that Rad51c should be considered a Trp53-dependent tumor suppressor. However, we cannot rule out the possibility that there are strain-specific modifiers linked to the originating alleles of the Trp53 and Rad51c knockouts that, when combined, alter the tumor spectrum in DH-cis and DH-trans mice.

Why are Rad51c tumors tissue specific? Our findings raise two important questions: How does loss of Rad51c give rise to malignancies that are tissue specific in spite of its role in DNA repair in every cell? How does addition of Rad51c-deficiency prevent development of Trp53-characteristic tumors in compound heterozygotes? With regard to the first question, examples of tissue-specific tumors caused by defects in genes that are essential for every cell type are well known. Genes like BrcA1, BrcA2, and Fbxw7/Cdc4; refs. 41–44). Although tumor latency in DH-cis mice is primarily determined by the LOH at the Trp53 locus, additional loss of Rad51c essentially overrides the tissue-specific effect of Trp53 mutation and promotes a shift from sarcomas (mesodermal origin) to malignancies of skin and adnexa (epidermal origin), especially those of specialized sebaceous glands and other glandular and neuroepithelial tissues such as those in the nasal and pericircular area. Therefore, we conclude that Rad51c should be considered a Trp53-dependent tumor suppressor. However, we cannot rule out the possibility that there are strain-specific modifiers linked to the originating alleles of the Trp53 and Rad51c knockouts that, when combined, alter the tumor spectrum in DH-cis and DH-trans mice.

How Rad51c deficiency can prevent development of Trp53-characteristic tumors such as to osteosarcomas and muscle sarcomas is unclear (Table 2). One possibility is that LOH for Rad51c may interfere with survival, differentiation, or neoplastic transformation of the cells that give rise to these tumors. This is supported by the observation that one of the six osteosarcoma samples from DH-cis females shows LOH only for Trp53 and not for Rad51c (Fig. 3D, see T11a). Interestingly, a myoepithelioma in the salivary gland of the same animal exhibits loss of wild-type alleles of both Rad51c and Trp53. Identification of the cell types that give rise to osteosarcomas and muscle sarcoma and the role of Rad51c in these cells will help understand the cause of reduction of these tumor types in DH-cis mice.

Tumors of specialized sebaceous glands in DH-cis mice. The high frequency of tumors in specialized sebaceous glands in DH-cis mice is unique and unexpected. Preputial gland tumors are rare in rodents (2.9% frequency reported for rats and even less for mice; ref. 48) and have been reported to develop in only a few mouse models (49, 50). In humans, 75% of sebaceous tumors arise in the periocular region, often from a specialized sebaceous gland of the eyelid, the meibomian gland (similar to DH-cis mouse tumor shown in Supplementary Fig. S1E; refs. 51, 52). This type of malignancy is rare but aggressive and represents 1% to 5.5% of eyelid malignancies (52). A small portion of sebaceous tumors is associated with Muir-Torre syndrome (53). The etiology of sebaceous cancers is unclear, but most MTS patients were found deficient in the mismatch repair pathway, primarily due to loss of MSH2 protein (54). In tumors from DH-cis mice we did not find any loss or down-regulation of Msh2 or microsatellite instability, suggesting that the mismatch repair mechanism is not involved.

Partial lethality of Rad51c<sup>ko/ko</sup>: haploinsufficiency or a modifier effect? Some Rad51c<sup>ko/ko</sup> embryos also had an abnormal phenotype and the ratio of viable Rad51c<sup>ko/ko</sup> mice was sub-Mendelian relative to the wild-type. Haploinsufficiency is unlikely to be the cause of partial Rad51c<sup>ko/ko</sup> lethality because no lethality was observed previously for Rad51c<sup>ko/ko</sup> mice expressing only 15% of the normal protein level (19, 38–40). We speculate that the presence of genetic modifiers may play a role. It is possible that one or several alleles cosegregating with the mutant allele of Rad51c may have overtly affected survival.

In conclusion, our results suggest that Rad51c functions as a tumor suppressor in mice. This is the first demonstration of a role in tumorigenesis for any Rad51 family member in mice. Similar studies may reveal unexpected tissue-specific effects for other Rad51 family members. Our future studies will be focused on examining the role of Rad51c in human tumors and on understanding the tissue-specific functions of Rad51c in epithelial tissues.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References

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