Palb2 synergizes with Trp53 to suppress mammary tumor formation in a model of inherited breast cancer

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.1073/pnas.1305362110</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:29070114">http://nrs.harvard.edu/urn-3:HUL.InstRepos:29070114</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Palb2 synergizes with Trp53 to suppress mammary tumor formation in a model of inherited breast cancer


Department of Cancer Biology, and Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA 02215; Cancer Institute of New Jersey, New Brunswick, NJ 08901; Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08854; Division of Molecular Pathology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; Biostatistics, Harvard School of Public Health, Boston, MA 02115; Department of Pathology, Brigham and Women’s Hospital, Boston, MA 02115; Rodent Histology Core, Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, MA 02115; and Laboratory of Genome Integrity, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Contribution by David M. Livingston, March 21, 2013 (sent for review February 20, 2013)

Germ-line mutations in PALB2 lead to a familial predisposition to breast and pancreatic cancer or to Fanconi Anemia subtype N. PALB2 performs its tumor suppressor role, at least in part, by supporting homologous recombination-type double strand break repair (HR-DSBR) through physical interactions with BRCA1, BRCA2, and RAD51. To further understand the mechanisms underlying PALB2-mediated DNA repair and tumor suppression functions, we targeted Palb2 in the mouse. Palb2-deficient murine ES cells recapitulated DNA damage defects caused by PALB2 depletion in human cells, and germ-line deletion of Palb2 led to early embryonic lethality. Somatic deletion of Palb2 driven by KI4-Cre led to mammary tumor formation with long latency. Codelivery of both Palb2 and Tumor protein 53 (Trp53) accelerated mammary tumor formation. Like BRCA1 and BRCA2 mutant breast cancers, these tumors were defective in RAD51 focus formation, reflecting a defect in Palb2 HR-DSBR function, a strongly suspected contributor to Brca1, Brca2, and Palb2 mammary tumor development. However, unlike the case of Brca1-mutant cells, Trp53bp1 deletion failed to rescue the genomic instability of Palb2- or Brca2-mutant primary lymphocytes. Therefore, Palb2-driven DNA damage control is, in part, distinct from that executed by Brca1 and more similar to that of Brca2. The mechanisms underlying Palb2 mammary tumor suppression functions can now be explored genetically in vivo.

mouse model | familial breast cancer

Partner and Localizer of BRCA2 (PALB2) is a breast cancer susceptibility gene. Its product was identified as a major interacting protein of the Breast Cancer susceptibility gene product 2, BRCA2 (1). This interaction is required for the repair of DNA double strand breaks (DSBs) by homologous recombination (HR) because PALB2 is necessary for the chromatin association of BRCA2 and its partner, RAD51 (1). RAD51 is the central recombinase in HR, and it participates in D-loop formation and strand displacement (2). PALB2 also plays a BRCA2-independent role in the HR process by enhancing RAD51 function (3, 4). PALB2 interacts with both BRCA1 and BRCA2 and mediates the long-known interaction between these proteins (5, 6). Loss of PALB2 does not affect BRCA1 recruitment to irradiation-induced foci (IRIF) but abrogates colocalization of BRCA2 and RAD51 at these structures (1, 5). Genetic analyses have shown that, like BRCA2, a member of Fanconi anemia complementation group D1, PALB2 is also the Fanconi anemia complementation group N protein (FANCN) (7, 8). PALB2 is also a breast cancer suppressor protein in its own right (9–12). Unlike BRCA1 and BRCA2 mutant tumors, only some PALB2-associated breast cancers have undergone loss of PALB2 heterozygosity (LOH) (9, 10). This finding implies that a reduction of PALB2 gene copy number might be sufficient to allow breast cancer development in some, but not all, settings. Why this difference exists is an open question.

Breast cancer in PALB2-mutated families is of intermediate penetrance, unlike in BRCA1/2 families (10, 12). Although PALB2 mutations are rarer than BRCA1/2 mutations, available clinical data suggest that heterozygous, germ-line PALB2 mutations do not precisely phenocopy either BRCA1 or BRCA2 predisposition syndromes (9, 10). This finding is consistent with the notion that PALB2 biological functions extend beyond simply enabling BRCA1–BRCA2 complex formation. PALB2 also interacts with BRCA1 (9, 10) and with BRCA1-RAD51 complex formation, but BRCA1 and BRCA2 are known partners and of the ChAM domain to the BRCA1-PALB2-BRCA2 HR machinery and/or to PALB2’s cancer suppression function is unclear. Thus, it is conceivable that PALB2 exerts multiple functions that extend beyond its known role in HR-mediated double strand break repair.

To date, it has been difficult to study the molecular pathogenesis of PALB2 breast cancer in detail because of the lack of a genetically engineered mouse model that recapitulates the human disease. Thus, we have generated a model of Palb2 breast cancer in the mouse and have documented its most salient properties. An analogous model was recently generated by others (16).

Results and Discussion

Targeting the Mouse Palb2 Gene and Generation of Palb2-Deficient ES Cells. To generate a Palb2 allele that could be conditionally inactivated upon Cre recombinase expression, we insertedloxP sites flanking exons 2 and 3 of the Palb2 gene (Fig. S1 A and B). These exons encode a putative nuclear localization signal sequence and the PALB2 coiled-coil domain (Fig. L1, Fig. S1L). The latter mediates the PALB2 interaction with BRCA1 (5, 6). Deletion of these exons would result in out-of-frame reading of exon 4 and premature termination of the PALB2 translation before the BRCA2-interacting, seven-bladed WD40-type β-propeller domain (Fig. L1, Fig. S1L). Due to premature truncation


The authors declare no conflict of interest.

1Present address: Department of Bioinformatics and Computational Biology, Genentech, South San Francisco, CA 94080.

2Present address: Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

3To whom correspondence may be addressed. E-mail: chryssia.kanellopoulou@nih.gov or david_livingston@dfci.harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1305362110/-/DCSupplemental.
ORF, the resulting transcript is also a candidate for Palb2 and cells (Fig. 1). To test whether the conditional gene targeting approach that was used had generated an allele that would be rendered null after Cre action, the response of Palb2-null and Palb2−/+ ES cells to DNA damaging agents that cause double strand breaks was analyzed. Normally, exposure of PALB2-proficient cells to ionizing radiation (IR) leads to the formation of phosphorylated histone H2AX (γH2AX) nuclear foci and subsequent recruitment of BRCA1, BRCA2, and RAD51 to these structures. As expected, after exposure to IR, γH2AX and BRCA1/IRIF formation was unaffected in Palb2−/+ cells (Fig. 1C, Fig. S2A). However, the recruitment of RAD51 was severely compromised (Fig. 1C). This defect was also evident at the biochemical level because no increase in chromatin loading of RAD51 after IR could be detected in nuclear extracts of IR-treated Palb2−/+ cells (Fig. 1D).

Because biallelic PALB2 mutations in humans cause Fanconi anemia, a hallmark of which is increased sensitivity to DNA cross-linking agents such as mitomycin C (MMC), the sensitivity of Palb2−/+ ES cells to MMC as well other DNA damaging agents was assayed. Both Palb2−/+ ES lines displayed increased sensitivity to MMC, IR, and the radiomimetic drug, neocarzinostatin (Fig. 1E, Fig. S2 B and C). These findings further imply that these Palb2−/+ cells are functional KOs for Palb2 because they are compromised in multiple, known Palb2-associated functions. Thus, upon Cre-mediated recombination in vivo, the aforementioned conditional Palb2 allele appears to be converted to a Palb2-null allele.

Loss of Palb2 in the Germ Line Results in Early Embryonic Lethality. Germ-line deletion of Brca1 or Brca2 results in early embryonic lethality (17, 20, 21). Although Palb2−/+ ES cells displayed no apparent growth defects compared with Palb2+/− controls, Palb2−/− loss could still be deleterious in differentiated progeny cells, and thereby negatively affect mouse development. Indeed, we were unable to obtain Palb2−/− mice from heterozygous crosses (Fig. S3A), consistent with previous reports (19, 22). Dissection of embryos from timed pregnancies revealed that Palb2-null embryos could be recovered only up to E12.5, but even then at sub-Mendelian ratios. These embryos repeatedly exhibited severe malformations. At earlier time points, morphological aberrations of Palb2−/− embryos were less obvious. However, these mutant embryos were clearly smaller than WT or heterozygous littermate embryos (Fig. S3B), and some displayed exencephaly as well as malformations of the placental labyrinth and yolk sac-associated blood islets (Fig. S3 C–F). The fact that Palb2 nullizygosity resulted in embryonic lethality detectable at E8.5–E10.5 is consistent with earlier reports showing that homozygous Palb2-deficient mice also die during embryogenesis at ∼E8.5 (19, 22).

Embryonic lethality due to loss of Brca1 or Brca2 can be delayed by concomitant loss of P53 (encoded by Trp53) or the CDK inhibitor p21 (encoded by Cdkn1a gene) (20, 24). Ttp53 loss also delayed the lethality of Palb2 KO embryos, which otherwise exhibited increased p21 abundance (22). We therefore tested whether loss of p21 expression affects Palb2−/− embryonic lethality by generating Palb2−/−; Cdkn1a−/+ embryos. As expected, loss of p21 expression did delay embryonic lethality of Palb2 KO embryos by 2–3 d (Fig. S3A). However, all Palb2−/−; Cdkn1a−/+ KO embryos still displayed multiple malformations and impaired growth compared with Palb2 heterozygous or WT littermates.
and were eventually resorbed. Therefore, these Palb2<sup>fl/fl</sup> embryonic rescue effects were incomplete and failed to suppress embryonic lethality.

Because the establishment of the placenta and onset of embryonic hematopoiesis are critical steps in development that take place around the time of lethality of Palb2 embryos, we asked whether the lethality of Palb2 KO embryos could be bypassed by a WT placenta. To this end, we used the Palb2<sup>fl/fl</sup> and Palb2<sup>−/−</sup> ES cells we had generated to perform tetraploid complementation assays. In this assay, diploid KO ES cells are aggregated to tetraploid WT blastocysts to generate KO embryos that are supported by a WT placenta because the tetraploid WT blastomeres are still capable of forming a placenta but cannot contribute to the embryo.

We found that embryos derived from the Palb2<sup>−/−</sup> ES cells were already underdeveloped and malformed at E9.5 compared with their Palb2<sup>fl/fl</sup> counterparts (Fig. S3 I and J). At E12.5, embryos derived from Palb2<sup>fl/fl</sup> ES cells appeared normal whereas embryos from Palb2<sup>−/−</sup> ES cells had been resorbed (Fig. S3 K and L). Likewise, breeding of the Palb2<sup>fl/fl</sup> conditional allele to Meox2-Cre knock-in (K1) mice (in which Cre is expressed from the endogenous Meox2 locus only in the embryo proper and not in the placenta or extraembryonic tissues) (25) only yielded viable mice in which the Palb2 deletion was incomplete. Collectively, these findings indicate that Palb2 is an essential gene during development, and its deficiency in the embryo proper is incompatible with life. These findings are analogous to previous results showing that the lethality of Brea1<sup>−/−</sup> embryos could not be rescued by tetraploid complementation assay (20).

**Palb2 is a Breast Tumor Suppressor in Mice.** To assess the effect of Palb2 loss-of-function on mammary tumorigenesis, we crossed Palb2<sup>fl/fl</sup> mice with keratin 14 promoter-driven Cre (K14-Cre) transgenic mice (26). K14-Cre transgenic animals preferentially express Cre recombinase in the basal epithelium of the mammary ducts, as well as in skin and oral mucosa. K14-Cre has previously been used to model murine Brea1 and Brea2 mammary tumorigenesis (27).

PALB2, like BRCA1 and -2, appears to be a breast cancer suppressor in humans (9, 10, 28). Therefore, in an effort to develop a tractable system for studying how Palb2 operates in this regard, we set out to develop a Palb2 mouse breast cancer model. Mammary tumor formation initiated by BRCA1 or BRCA2 loss requires concomitant loss of functional p53 (encoded by Trp53 in mice) (27, 29). This observation was considered in efforts to establish a Palb2 model.

We first generated Palb2<sup>fl/fl</sup>/Trp53 double conditional mice by crossing Palb2<sup>fl/fl</sup>, K14-Cre transgenic mice with Trp53 conditional normal mice. All mice that harbored the conditional alleles for Palb2 and/or Trp53 in the absence of K14-Cre were phenotypically normal, fertile, and capable of nursing their litters. During the period of tumor monitoring (up to 600 d after birth), Trp53<sup>−/−</sup>; K14-Cre female mice developed spontaneous mammary tumors with a frequency of ~80% and a mean tumor-free interval (T<sub>D</sub>) of 320 d. By contrast, Palb2<sup>fl/fl</sup>; Trp53<sup>−/−</sup>; K14-Cre double conditional mice developed tumors much faster (T<sub>D</sub> = 192 d, P = 2.4 × 10<sup>-5</sup>), indicating that Palb2 loss accelerates tumor formation on a Trp53<sup>−/−</sup> background (Fig. 2A). These latencies are comparable with Brea2<sup>−/−</sup>; Trp53<sup>−/−</sup>; K14-Cre and Brea1<sup>−/−</sup>; Trp53<sup>−/−</sup>; K14-Cre mice (T<sub>D</sub> = 181 and 213 d, respectively) (27, 30).

Somatic loss of one Trp53 allele displayed, as expected, a haploinsufficient tumor suppressor phenotype (27, 30), given that Palb2<sup>fl/fl</sup>; Trp53<sup>−/+</sup>; K14-Cre mice developed tumors significantly faster than Palb2<sup>fl/fl</sup>; Trp53<sup>−/−</sup>; K14-Cre mice (T<sub>D</sub> = 225 d vs. 420 d, respectively, P = 2.5 × 10<sup>-12</sup>; Fig. S4C). Palb2 loss of function also accelerated tumor formation on a Trp53<sup>−/−</sup> heterozygous (H+/−) background, again reflecting the genetic interaction of these two genes (Fig. 2B).

K14-Cre-mediated loss of Palb2 and Trp53 led, predominantly, to tumor formation in breast, skin, and oral mucosa (Fig. 2E), as previously reported for K14-Cre-driven Brea1 and Brea2 cancers (27, 30–32). All of the mammary cancers were estrogen and progestosterone receptor (ERPR)-negative, basal-like (Fig. S4 D–F), much like the Brea1 and -2 tumors generated by K14-Cre (30, 32). Although most of the tumors found in Palb2<sup>−/−</sup>; Trp53<sup>−/−</sup>; K14-Cre mice were breast carcinomas, Palb2<sup>−/−</sup>/Trp53<sup>−/−</sup> compound KO mice displayed an expanded spectrum of tissues affected by tumors (Fig. 2E), suggesting that combined loss of PALB2 and P53, possibly due to some expression of Cre in other tissues, also results in tumor formation that is not restricted to the mammary gland.

Mice harboring conditional alleles for Palb2 and Trp53, but no K14-Cre transgene, and Palb2<sup>−/−</sup>; Trp53<sup>−/+</sup>; K14-Cre mice did not display overt tumor formation during the observation period (Fig. 2C), despite the intrinsic mutagenic activity of Cre in mammalian cells (31, 33). Therefore, tumor formation in the above-noted experiments is a product of targeted gene deletion.

All tumors from Palb2<sup>−/−</sup>; Trp53<sup>−/−</sup>; K14-Cre mice (n = 12) had lost both copies of Palb2 and Trp53 (Fig. 2D). Similarly, in all tumors from Palb2<sup>−/−</sup>; Trp53<sup>−/+</sup>; K14-Cre mice (n = 15), the conditional Palb2 and Trp53 alleles were recombined. The WT copy of Trp53 was also lost in most tumors, probably through LOH.

Early reports describing a lack of PALB2 LOH in clinical tumor samples from heterogeneous patients suggested that PALB2 could be a haploinsufficient tumor suppressor in humans whereas other reports showed that multiple PALB2 tumors revealed PALB2 LOH, implying that the PALB2 tumor formation process is not uniform (9, 34, 35). In our experimental setting, no haploinsufficiency for tumor suppression was observed for Palb2, as indicated by the comparable latency in Palb2<sup>fl/fl</sup>; Trp53<sup>−/−</sup>; K14-Cre and Palb2<sup>−/−</sup>; Trp53<sup>−/−</sup>; K14-Cre tumor development (P = 0.46, Fig. S4A). Similarly, when compared on a Trp53<sup>−/−</sup>; K14-Cre background, cohorts of Palb2<sup>−/−</sup> and Palb2<sup>−/−</sup> mice developed tumors with similar latency and frequency (P =
0.96, Fig. S4B), implying that heterozygous *Palb2* loss of function did not contribute to tumor formation. Moreover, *Palb2* heterozygous mouse breast tumor lines displayed proper RAD51 localization at IRIF, consistent with preserved HR function (see Fig. 4C). By contrast, *Palb2*-null breast tumor cell lines displayed the same defect in RAD51 accumulation observed in *Palb2*-null primary cells (see Fig. 4C). These findings suggest a role for HR deficiency in the genesis of *Palb2* tumors, a state that is not compatible with retention of a functional copy of the gene. Moreover, an analysis of tumors that arose in *Palb2*+/−; *Trp53*fl/fl, K14-Cre mice implies that a significant fraction of these tumors retained at least one copy of *PALB2*, suggesting that loss of one copy of the gene did not contribute to tumor formation in this model (Fig. 2D).

Although we observed long latency tumors only in *Palb2*fl/fl; K14-Cre mice on a WT *Trp53* background (T1/2 = 420 d), tumor formation was nonetheless highly significant compared with *Palb2*+/−; *Trp53*+/−; K14-Cre controls (P = 5.4 × 10−26, Fig. 2C). The majority of these tumors were small lesions in the head and neck, and a few were mammary tumors. All of these mammary tumors were *Palb2*−/−, and all displayed either mutations in or loss of *Trp53* by LOH.

The finding that loss of *Palb2* alone is sufficient to induce long latency tumor formation contrasts with most *Brca1* and *Brca2* mouse models in which significant numbers of these tumors could not be detected, unless *Trp53* was codeleted (27, 30, 36–37). One explanation for this finding is that somatic *Palb2* loss might be better tolerated than somatic *Brca1* loss on a *Trp53* WT background. This hypothesis fits with the finding that *Palb2* nullizygosity gives rise to a less severe phenotype in ES cells than biallelic *Brca1* or *Brca2* loss (17, 18, 27, 38).

**Genomic Features of *Palb2*/*Trp53*-Deficient Mammary Tumors.** Genomic instability is a hallmark of human cancer, and it promotes tumor initiation and progression. Experimental mouse tumor models have recapitulated this aspect of human tumorigenesis (39). To gain insight into the genomic structures of the tumors that arose due to the loss of *Palb2*, we performed high-resolution comparative genomic hybridization (CGH) (40) analysis of *Palb2*/*Trp53*, *Brca1*/*Trp53*, *Brca2*/*Trp53*, and *Trp53* only-deficient mammary tumors that arose in K14-Cre mice (Fig. 3A–C).

Segmentation analysis of the CGH data was performed for each tumor to assess the number of genomic segments with deviating copy number changes (aka genomic segmentation), as a readout of genomic instability (41). *Palb2*/*Trp53* and *Brca1*/ *Trp53* tumors displayed apparently greater genomic instability (genomic segmentation) compared with *Brca2*/*Trp53* and *Trp53*-only tumors, but the difference was not statistically significant. However, when the relative dose of amplified segments (log2 dose > 0.5) was analyzed, *Palb2*/*Trp53* mammary tumors (n = 8) displayed a significantly higher average dose of amplified segments than either *Brca1*/*Trp53* (n = 4; P < 0.0001, Fig. 3C) or *Brca2*/*Trp53* tumors (n = 5; P = 0.0014, Fig. 3C). The low numbers of deletions (log2 dose ≤ −0.5) detected in *Brca2*/*Trp53* and *Trp53*-only tumors precluded further analysis of this aspect of genomic instability (Fig. 3B).

No significant difference in focal genome amplification appeared when *Palb2*/*Trp53* tumors were compared with *Trp53*-only tumors, indicating that *Palb2*/*Trp53* tumors share a similar amplification-prone genomic profile with *Trp53*-only tumors, despite their marked difference in tumor formation kinetics. The inability of *Palb2* loss to suppress focal genomic amplifications (unlike what was observed in *Brca1*/*Trp53* and *Brca2*/*Trp53* tumors) could be accounted for by three, alternative explanations.

First, these differences could be due to residual activity of the conditional *Palb2* allele we generated, and other *Palb2* loss of function mutations might trigger the formation of true *Brca2* tumor phenocopies. Second, our allele is a functional null, as our studies suggest, but complete loss of *Palb2* is similar to a BRCA2 hypomorphic phenotype rather than a complete loss of BRCA2 function. Alternatively, there are PALB2 functions that are, at least in part, nonoverlapping with the tumor suppressing functions of its BRCA2 partner protein. New experiments with additional *Palb2* and *Brca2* mutant mouse strains would be required to distinguish between these possibilities.

Finaly, tumor heterogeneity likely affected the CGH profiles (Fig. S5) in ways that make it difficult to identify regions of chromatomal imbalances that were unique to *Palb2*/*Trp53* tumors. Conceivably, a more comprehensive analysis with a much larger collection of tumor samples would reveal such regions.

---

**Loss of 53BP1 Fails to Rescue the HR Defect Caused by PALB2 Deficiency.** Loss of 53BP1 can rescue the HR defect and lethality observed in either *Brca1Δ1/Δ1* or *Brca1-null* cells and mice (42–45). Similarly, decreased expression of the P53 binding protein 1 (53BP1) was detected in triple negative breast cancers as well as human *BRCA1* tumors (45). Therefore, we asked whether *Trp53bp1* (which encodes mouse 53BP1) expression is reduced in *Palb2*/*Trp53* KO tumors, and whether its absence rescues the HR defect associated with *Palb2* loss. Quantitative RT-PCR analysis of *Trp53bp1* mRNA in freshly isolated *Palb2* breast tumor samples
showed that levels of Trp53bp1 mRNA in freshly isolated tumor samples that are either Palb2-proficient (+/+ and +/-, n = 8) or Palb2-deficient (−/−, n = 14) varied considerably (Fig. S6B). The number of chromosomal aberrations observed was not significantly different in Palb2-deficient and Palb2-proficient tumors (Fig. 4A).

To determine whether HR deficiency due to Palb2 loss is complemented by Trp53bp1 loss in primary cells (cultured primary splenic B cells), we generated Palb2+/fl; CD19-Cre mice that were or were not deficient in Trp53bp1. Cultured primary splenocytes from these mice were then assayed for HR competence upon treatment with PARP inhibitors (PARPi), which selectively induces DNA damage and chromosome aberrations in HR-deficient cells (46). Treatment with KU0058948 (PARPi) led to an accumulation of chromosomal and chromatid breaks, and radial structures were evident in chromosomal spreads from cultured Palb2+/fl; Trp53bp1−/−; CD19-Cre primary splenocytes (Fig. 4B). The number of chromosomal aberrations observed was not reduced in Palb2+/fl; Trp53bp1−/−; CD19-Cre splenocytes, implying that Trp53bp1 deletion did not complement the HR defect caused by Palb2 deficiency (Fig. 4B). Trp53bp1 deletion also failed to rescue the chromosomal aberrations found in spreads from PARPi-treated Brca2−/−; Trp53bp1−/−; CD19-Cre splenocytes (Fig. S6A), which appeared to be even more extensive than those observed in PARPi-treated Palb2−/−; Trp53bp1−/−; CD19-Cre cells (Fig. 4B). As has been previously described (43, 45), complete rescue of the DNA repair deficiency in Brca1−/−; Trp53bp1−/−; CD19-Cre splenocytes was observed (Fig. S6B).

Of note, both Palb2/Trp53bp1 and Brca2/Trp53bp1 compound KO cells displayed more chromosomal aberrations after PARPi exposure than Palb2 or Brca2 single mutants (Fig. 4B, Fig. S6A). Thus, whereas Brca1, Palb2, and Brca2 manifest closely related, even overlapping functions, loss of Palb2 or Brca2 also resulted in a different DNA damage response after Trp53bp1 elimination from that manifested by Brca1 KO cells, in which Trp53bp1 codelletion rescued the genomic instability observed after PARP inhibition. These observations suggest that the contributions of PALB2 and BRCA2 to HR-based DSB repair are distinct from those of BRCA1 and cannot be complemented by 53BP1 loss. In keeping with existing evidence, PALB2 and BRCA2 may be described as HR effectors that cannot be replaced or bypassed, except by artificially forcing the loading of RAD51 onto chromatin at/near DSB, which 53BP1 loss has not yet been shown to promote (45, 47–49). These observations, along with earlier results (4), also suggest that PARP inhibition might be a potential therapeutic regimen in PALB2-deficient tumors, as it is in BRCA1- and BRCA2-associated tumors (46).

Conclusions

In summary, we have shown that Palb2 is a breast tumor suppressor gene in mice as it is in humans and that it synergizes with Trp53bp1 to suppress tumor formation. The outcome of dual Palb2/Trp53bp1 nullizygosity in the mouse mammary gland is highly penetrant breast cancer. In keeping with the fact that PALB2 is also a breast tumor suppressor in humans, PALB2 might be viewed as a BRCA3-like allele. Moreover, tumorigenesis driven by Palb2 loss in the mouse is not entirely suppressed on a Trp53 WT germ-line background, unlike most Brca1 and Brca2 mouse models of breast tumorigenesis (50).

Despite many similarities to Brca2/Trp53 and Brca1/Trp3 breast tumors, Palb2 tumors displayed certain divergent genomic features that might be viewed as separating them from BRCA1 and -2 cancers. Specifically, we observed patterns of genomic aberrations that were different in Palb2/Trp53-derived tumors from those detected in Brca1/Trp53- or Brca2/Trp53-derived tumors. These data are consistent with the hypothesis that PALB2 possesses biological functions that extend beyond those of its major interactors, BRCA1 and BRCA2. Alternatively, the effect of Palb2 deletion may mimic a phenotype akin to partial loss of BRCA2, resulting in a less dramatic genomic instability profile in the relevant tumor cells.

Although the genomic instability of Brca1-deficient cells can be rescued by loss of Trp53bp1, deletion of the latter had, if anything, an adverse effect in Palb2 KO cells. In that context, Palb2 is more similar to Brca2, the absence of which leads to an HR defect that also cannot be rescued by Trp53bp1 deletion. Haploinsufficiency for Palb2 tumor suppression was not detected in this model although one cannot rule out the possibility that it would be manifest in a different model system and/or with enlarged cohorts of experimental mice. For example, the tumors in this mouse model driven by K14-Cre were uniformly of the triple negative phenotype. This characteristic might well contribute to the absence of haploinsufficiency in our system, in the same way that BRCA1 mammary tumors derived from distinct cell populations display preferential patterns of consecutive LOH events along the tumorigenesis pathway (32, 51).

We believe that this mouse model will be useful in understanding how Palb2 serves its breast cancer suppression function.
Mice harboring tumors were humanely killed when the tumor diameter reached 2.0 cm in its greatest dimension. Mice that were otherwise severely diseased or distressed were also killed according to institutional guidelines. Mantel-Cox logrank test was applied for comparison of tumor-free survival of mouse cohorts.

**ACKNOWLEDGMENTS.** We thank Drs. Ron DePinho and William Kaelin for Trp53<sup>172+/-</sup> mice and Dr. Bing Xia for openly exchanging information on PALB2 KO mice with our laboratory. We also thank Drs. Kristine McKinney, Nana Naert-Keremey, Patrizia Dahlia, and Stefan Muljo for critical reading of this manuscript; Dvora Ghitza and Dr. Klaus Rajewsky for help with the tetraploid complementation assays; Dr. Rene Maeher for Vs.5 E5 ES cells; Dr. Ronny Drapkin for the antibody developed against mouse BRCA1; and James Horner for ES cell microinjections. We also thank all members of the D.M.L. laboratory for cooperation, expertise, and reagent sharing, as well as fruitful discussions. Finally, we thank the staff from the Dana-Farber Cancer Institute Animal Resources Facility for excellent technical support and Anuradha Kohli and Nancy Gerard for outstanding administrative support. This work was supported by National Cancer Institute Grant P01CA80111, by a Specialized Program of Research Excellence grant in breast cancer research (2P50CA089393) to the Dana-Farber/Harvard Cancer Center, by the Susan G. Komen Foundation for the Cure (SAC110022), and from the Breast Cancer Research Foundation.

**Materials and Methods**

**ES Cell Derivation, Embryo Harvesting, and Tetraploid Complementation Assay.** Generation of the conditional allele for Palb2 and additional experimental details are described in SI Material and Methods. Oligo sequences used are described in Table S1. Tetraploid and embryo tailing was performed according to standard protocols (S2). Pregnant mice from timed matings were killed at indicated time points by CO₂ asphyxiation following institutional guidelines. Uterine horns and embryos were dissected under the microscope, and isolated embryos were directly used for digestion, DNA extraction, and genotyping. Negative selection of Palb2 KO embryos was analyzed according to the Hardy–Weinberg equilibrium model, using an online tool (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Tetraploid complementation assays were performed as described (S3). All experimental procedures involving mouse work were approved by the Dana-Farber Cancer Institute Institutional Care and Use Committee under Animal Protocol 07-111.

**Tumorigenesis Studies.** Mouse cohorts were monitored for tumor formation biweekly. Mammary tumor formation was scored when a palpable tumor of 1.0 cm in its greatest diameter could be detected, as previously described (27, 30).


