Coiled-Coil Domain: Uncoiling Tumor Suppression by BRCA1

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The coiled-coil domain of BRCA1 is essential for its interaction with partner and localizer of BRCA2 (PALB2). In mice, loss of this interaction is known to result in Fanconi anemia–associated phenotypes. In a study published in this issue of *Cancer Research*, Pulver and colleagues from the Jonkers lab have generated a mouse model with a leucine to proline change in codon 1363 in the coiled-coil domain of BRCA1 (*Brca1*^{LP}), which disrupts its binding with PALB2. Unlike the previously reported viable coiled-coil defective mice, homozygous *Brca1*$^{LP/LP}$ mutant mice die during embryogenesis. The authors examined the role of the BRCA1/PALB2 interaction on mammary tumorigenesis and reported increased incidence of mammary tumors that are carcinosarcomas or sarcomatoids, unlike the adenocarcinomas that are characteristic mammary tumor types associated with loss of *Brca1* and *Trp53* in mice. The findings reveal the relevance of the coiled-coil domain in mammary tumor suppression by BRCA1.

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Each year more than a million women are diagnosed with breast cancer worldwide. Inheritance of pathogenic mutations in *BRCA1* and *BRCA2* increases the lifetime risk of developing breast cancer up to 72% and up to 44% for ovarian cancer (1). Breast tumors with a *BRCA1* mutation are predominantly basal-like and are classified as triple-negative tumors due to the lack of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression. Such tumors do not respond well to standard treatments, but they do respond well to PARP inhibitors (2). This is due to the fact that cells defective in homologous recombination (HR) exhibit synthetic lethality to PARP inhibition. BRCA1 is required for the repair of double-strand breaks (DSB) by HR. It plays an important role in the initial stages of HR by facilitating resection of 5′ ends of damaged DNA.

BRCA1, which consists of 1,863 amino acids with many binding partners, is considered to be a protein with multiple functions. In addition to the repair of DSBs by HR, it is involved in several other biological processes such as sensing of DSBs, cell-cycle regulation, chromatin remodeling, and R-loop resolution (3). BRCA1 also has E3 ligase activity, which is attributed to the C3-H-C4 type RING domain near the N-terminus. The two BRCT repeats near its C-terminus contribute to the transcriptional activation activity of BRCA1. These are the two key domains that are required for its function as a tumor suppressor, which is supported by the observation that most well-characterized pathogenic missense mutations are present in these two domains.

A few pathogenic variants have been identified that are located in the coiled-coil domain of BRCA1, which binds to the WD40 domain of PALB2. PALB2 is also a tumor suppressor and mutations in *PALB2* are associated with increased risk of breast cancer (3). PALB2 also binds to BRCA2 and plays a critical role in recruitment of BRCA2 to the chromatin to facilitate HR. Thus, PALB2 is functionally an important protein that connects to both BRCA1 and BRCA2 (3). Pulver and colleagues report here the generation and characterization of mice with a leucine to proline substitution in codon 1363 of BRCA1 (*Brca1*^{LP}), which is equivalent to the L1407P variant reported in human BRCA1 (4). This mutation has been shown to disrupt the binding of BRCA1 with PALB2. The functional significance of the interaction between BRCA1 and PALB2 is evident by the embryonic lethality of mice homozygous (*Brca1*$^{LP/LP}$) for the L1363P variant. Mutant embryos failed to survive beyond E11.5. This phenotype was milder than the early postimplantation lethality of *Brca1* null embryos (5). Similar to the *Brca1* null embryos, lethality of *Brca1*$^{LP/LP}$ embryos was delayed by a few days on a *Trp53*-deficient genetic background, but no viable *Brca1*$^{LP/LP}$ mice were obtained.

Mouse embryonic fibroblasts (MEF) generated from *Brca1*$^{LP/LP}$ embryos were found to be defective in HR as assessed by a severe reduction in ionizing radiation (IR)–induced RAD51 foci. Also, the cells were sensitive to cisplatin and PARP inhibitor AZD2461. BRCA1-deficient cells are defective in DSB end-resection, which is critical for progression of repair by HR (3). Interestingly, *Brca1*$^{LP/LP}$ MEFs exhibited normal end resection at DSBs, suggesting that the interaction between BRCA1 and PALB2 is not required for this initial step of HR. This interaction was also found to be dispensable for stability of stalled replication forks, which is consistent with the role of BRCA1-BARD1 interaction in replication fork stability (6).

The early embryonic lethality of *Brca1*$^{LP/LP}$ mice reported by Pulver and colleagues is in sharp contrast to mice carrying the same L1363P variant previously generated by Park and colleagues (**Fig. 1**; ref. 7). Although these mice were reported to be born at expected Mendelian ratio, both males and females were infertile and exhibited Fanconi anemia (FA)–associated phenotypes, such as growth retardation along with skeletal and other developmental abnormalities, and they succumbed to bone marrow failure or T-cell lymphoblastic lymphoma. A similar phenotype was reported by Nacson and colleagues in mice with a small in-frame deletion in the coiled-coil domain (*Brca1*^{CC}) that deleted residues 1361–1363, equivalent to 1405–1407 of human BRCA1 (**Fig. 1**; ref. 8). While a significant fraction of *Brca1*$^{CC/CC}$ mice died during embryogenesis, the survivors exhibited phenotypes consistent with those observed in patients with FA. Similar to the *Brca1*$^{LP/LP}$ reported by Park and colleagues, *Brca1*$^{CC/CC}$ mice were also smaller in size than their littermates, showed infertility, had decreased

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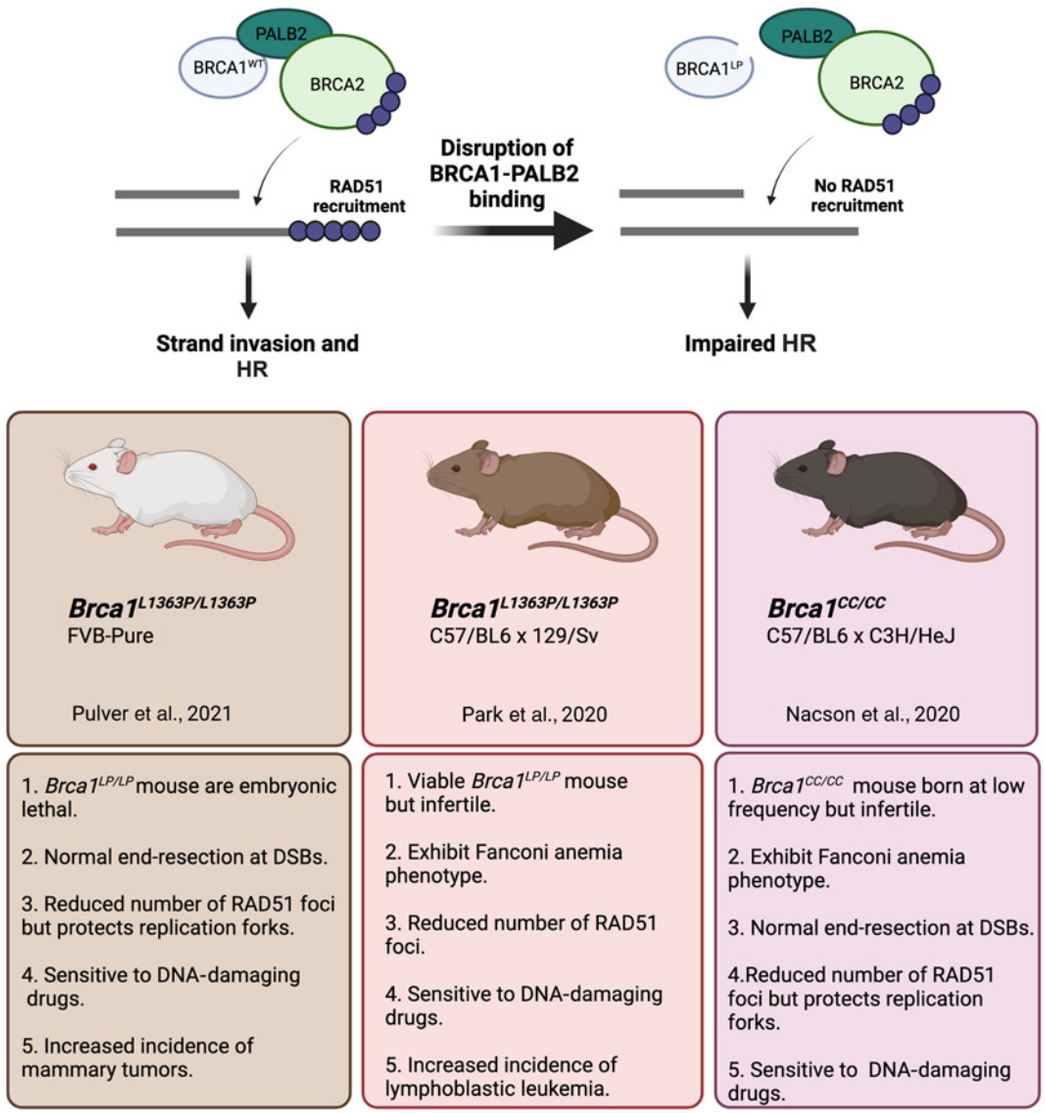

Figure 1.

Role of BRCA1 coiled-coil domain mediated interaction with PALB2 in HR. Top, schematic representation of the role of BRCA1-PALB2 interaction via the coiled-coil domain of BRCA1 in the recruitment of RAD51 by BRCA2 to the site of DSBs. Disruption in binding of BRCA1 to PALB2 either by a single amino acid substitution (leucine to proline in codon 1363) or deletion of three amino acids (1361–1363) in the coiled-coil domain has no impact on 5′ end resection but results in loss of IR-induced RAD51 recruitment and HR. Bottom, summary of the phenotype of the three *Brca1* mouse models with mutations in the coiled-coil domain.

erythrocytes and leukocytes, developed disseminated T-cell acute lymphoblastic leukemia, and died within 100 days. Both of the mouse models recapitulate the clinical features of patients with FA, which is consistent with the now established role of *BRCA1* and *PALB2* as FA genes. Although embryonic development is severely impacted in the model reported by Pulver and colleagues and no viable mice were obtained, the phenotypes at the cellular level are very similar to those observed in these two other models. The precise cause of the variability in the impact on embryonic development is unknown but a difference in their genetic backgrounds may be a contributing factor. Mice reported by Park and colleagues and Nacson and colleagues were on C57BL/6 × 129/Sv and C57BL/6J × C3H/HeJ mixed genetic backgrounds, respectively, and those generated by Pulver and colleagues are on a pure FVB background.

Pulver and colleagues also examined the impact of the coiled-coil domain on mammary tumorigenesis. Conditional deletion of *Brca1* and *Trp53* in epithelial cells using K14 promoter-driven *Cre recombinase* (*Cre*) expression is known to result in skin and mammary tumors (9). Using a similar strategy, the effect of the L1363P variant was examined in mice carrying one $Brca1^{LP}$ allele and one floxed allele of *Brca1*($Brca1^{F}$) along with floxed *Trp53* alleles and the *K14–Cre* transgene (*K14-Cre;*$Brca1^{LP/F}$;$Trp53^{F/F}$). These mice had a median tumor latency that was comparable with mice that were homozygous for the *Brca1* floxed allele (*K14-Cre;*

Brca1$^{F/F}$;*Trp53*$^{F/F}$). The incidence of mammary tumors in mice is known to be higher relative to skin tumors when both *Brca1* and *Trp53* are deleted compared with *Trp53* deletion. Interestingly, the incidence of mammary tumors increased and skin tumor incidence decreased in mice expressing the *Brca1*^{LP} allele. However, this difference in skin versus mammary tumor incidence was not evident on the *Trp53*$^{F/+}$ background. Pulver and colleagues also observed a marked difference in the histopathology of the tumors associated with *Brca1* loss and those expressing the *Brca1*^{LP} allele. The mammary tumors from *K14-Cre;Brca1*$^{F/F}$;*Trp53*$^{F/F}$ mice were predominantly adenocarcinomas but those from *K14-Cre;Brca1*$^{LP/F}$;*Trp53*$^{F/F}$ mice were carcinosarcomas with both epithelial and mesenchymal cells or mostly mesenchymal sarcomatoid tumors. The tumors with mostly mesenchymal features had less genomic instability, likely due to the presence of stromal cells that do not undergo K14-Cre–mediated loss of *Brca1* or *Trp53*. In spite of having a relatively more stable genome, the tumors from *K14-Cre;Brca1*$^{LP/F}$;*Trp53*$^{F/F}$ mice were sensitive to cisplatin and PARP inhibitor treatment, but the sensitivity was reduced compared with tumors deficient in *Brca1*. The level of sensitivity was consistent with the reduction in IR-induced RAD51 foci formation and severity in HR defect in tumor cells expressing the *Brca1*^{LP} allele.

The findings of Pulver and colleagues along with those reported by Park and colleagues and Nacson and colleagues have established a critical role for the coiled-coil domain of BRCA1 in embryogenesis as well as for normal growth and development (**Fig. 1**). The binding of BRCA1 with PALB2 via this domain may be dispensable for the stability of stalled replication forks and 5′ end resection at DSBs, but it is essential for RAD51 recruitment. These studies also provide evidence to support the significance of the coiled-coil domain in BRCA1-mediated tumor suppression.

The physiological significance of the BRCA1-PALB2-BRCA2-RAD51 axis remains to be fully understood. Functionally, the interaction between these proteins is clearly essential for HR. Loss of any of these proteins individually or mutations disrupting the binding domains of any of these proteins have a severe impact on RAD51 recruitment at DSBs, yet the phenotypes observed in mice carrying these alterations vary widely. Mice harboring a mutation in *Palb2* that disrupts its binding with BRCA1 are viable and the only overt phenotype observed is male infertility (10). In humans, the cancer subtypes associated with *BRCA1*, *BRCA2*, and *PALB2* mutations are also different. Clearly, a lot remains to be learned about the biological functions of these proteins and their binding partners to fully understand how they contribute to tumor suppression.

Authors' Disclosures

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