PALB2/FANCN: Recombining Cancer and Fanconi Anemia

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Abstract

Partner and localizer of BRCA2 (PALB2) was originally identified as a BRCA2-interacting protein that is crucial for key BRCA2 genome caretaker functions. It subsequently became clear that PALB2 was another Fanconi anemia (FA) gene (FANCN), and that monoallelic PALB2 mutations are associated with increased risk of breast and pancreatic cancer. Mutations in PALB2 have been identified in breast cancer families worldwide, and recent studies have shown that PALB2 also interacts with BRCA1. Here, we summarize the molecular functions and clinical phenotypes of this key DNA repair pathway component and discuss how its discovery has advanced our knowledge of both FA and adult cancer predisposition. Cancer Res 70(19); 7353–9. ©2010 AACR.

Identification and Functional Studies of PALB2

Partner and localizer of BRCA2 (PALB2) was identified by searching for novel components of endogenous BRCA2-containing complexes (6). The PALB2 gene consists of 13 exons and maps to chromosome 16p12.2 (Fig. 1A), a region that shows loss of heterozygosity in around 12% of breast cancers (7). The 1,186–amino acid protein has a coiled-coil motif at the N terminus and a C-terminal domain containing a series of WD repeats (Fig. 1B). The protein was found to be associated with around 50% of cellular BRCA2 and is critical for its chromatin localization and recruitment to DNA damage sites (6). Like BRCA2-deficient cells, PALB2-knockdown cells exhibited diminished HR activity, MMC sensitivity, and intra-S-phase checkpoint defects. Later, it was found that PALB2-deficient FA-N cells lacked chromatin-bound BRCA2 and were completely unable to form RAD51 foci (8). The PALB2 binding site was mapped to the extreme N terminus of BRCA2, which is both necessary and sufficient for the binding. Importantly, eight naturally occurring, breast cancer patient–derived BRCA2 unclassified variants were found to exist within or very close to the PALB2 binding region, and functional analyses of the variants showed that three of the eight variants disrupted PALB2 binding, and the same three (and only the same three) variants also abrogated BRCA2 HR function (6). Together, the strong correlation between the ability of a BRCA2 variant to bind PALB2 and its ability to support HR, and the complete lack of RAD51 foci in PALB2-deficient cells indicate that PALB2 is crucial for BRCA2 HR function. To the extent that the HR function of BRCA2 is generally believed to be essential for its tumor suppression activity and that the three mutants are derived from breast cancer patients, the above findings further suggest that PALB2 is important for BRCA2-mediated tumor suppression. Similar to Brca2, homozygous Palb2 knockout in mice causes embryonic lethality, and heterozygous animals are normal (9).

Like BRCA2, BRCA1 has also been known to be important for HR, and the two breast cancer proteins coexist in an endogenous protein complex (10). Yet, how these two proteins
Figure 1. The BRCA complex of HR repair and tumor suppression. A, the PALB2 gene locus in chromosome 16p12.2. The image is generated using the NCBI Sequence Viewer and slightly modified. B, schematic of the PALB2 protein structure showing its domains responsible for binding with BRCA1, BRCA2, and MRG15. C, a proposed model of BRCA complex assembly at sites of DNA double-strand breaks. It is currently unclear which BRCA1 BRCT domain-binding partners (BRIP1/FANCJ, CCDC98-RAP80, or CtIP) exist in the core BRCA1/PALB2/BRCA2 complex. Also, BRCA1 may be recruited to damage sites via two distinct mechanisms—one by interacting with the MRE11/RAD50/NBS1 (MRN) complex and the other via its binding to the CCDC98/RAP80 complex—and it remains to be seen which branch is responsible for PALB2/BRCA2/RAD51 recruitment.
associate with each other, and whether they work together in HR, remained unknown until recently. From three studies published in close succession, it was established that PALB2 physically links BRCA1 and BRCA2 to form a “BRCA complex” (11–13). Specifically, a coiled-coil motif in the N terminus of PALB2 directly binds another coiled-coil motif in the “pre-BRCT” domain of BRCA1, which was exactly the BRCA1 domain originally found to be responsible for BRCA2 binding (10). In contrast, PALB2 directly interacts with BRCA2 with its C-terminal WD repeats domain, whose crystal structure, a seven-bladed β-propeller, has been solved in a complex with the cognate BRCA2 N-terminal peptide (14). It was also found that PALB2 and BRCA2 focus formation was largely abolished in BRCA1-mutant HCC1937 cells and that acute knockdown of BRCA1 abrogated endogenous PALB2/BRCA2 foci (11, 13). Furthermore, several point mutations in PALB2 specifically disrupting BRCA1 binding were generated, and all resulted in a failure of the protein to support HR (12, 13). Finally, multiple clinically relevant point mutations in the coiled-coil domain that abolish PALB2 binding were identified in BRCA1 and were shown to disable its HR function (12). Taken together, these findings strongly supported the notion of a BRCA1-PALB2-BRCA2-RAD51 pathway critical for the initiation of HR and suppression of cancer and FA (Fig. 1C).

However, two significant discrepancies have emerged with respect to the regulation of PALB2 function. First, it remains unclear if PALB2 recruitment to DNA damage sites is strictly dependent upon BRCA1. Two of the three studies above showed that endogenous PALB2 failed to form clear foci in the absence of BRCA1 (11, 13), but the third showed that ectopically expressed PALB2 point-mutant proteins largely unable to bind BRCA1 were still able to form foci with nearly normal efficiency (12), raising the question of whether PALB2 may be able to form two distinct types of nuclear foci, one dependent and one independent of BRCA1. Second, the MORF-related protein, MRG15, which is a component of certain chromatin remodeling complexes, has been identified as a major PALB2 binding partner (15). In the same study, it was found that downregulation of MRG15 leads to an increase of HR and sister chromatid exchange (SCE), suggesting that MRG15 may restrict HR, be it through PALB2 or not. But a new study, which independently identified the MRG15-PALB2 interaction, presents evidence that MRG15 may, in fact, facilitate HR by promoting PALB2 chromatin localization (16). Further studies will be necessary to clarify or reconcile these conflicting observations.

**PALB2 and Fanconi Anemia**

Immediately after PALB2 was discovered, biallelic pathogenic mutations were identified in eight FA-N families (8, 17). In some respects, FA-N cases arising in PALB2 biallelic mutation carriers have a typical FA phenotype with growth retardation and variable congenital malformations. However, PALB2-related FA is associated with an unusually severe predisposition to pediatric malignancies (Table 1), with all eight described cases having developed cancer in early childhood, including five medulloblastomas, three Wilms tumors, two acute myelogenous leukemias, one neuroblastoma, and one kaposiform hemangioendothelioma (8, 17). The cancer spectrum for biallelic PALB2 mutation carriers is very similar to that of biallelic BRCA2/FANCD1 mutation carriers, who also are at high risk of embryonal tumors (18). The strong similarity of cancer types and ages of onset in FA for both PALB2 and BRCA2 biallelic mutation carriers again supports the proposition that PALB2 is important for BRCA2 tumor-suppression activity.

**PALB2 Mutations and Hereditary Susceptibility to Breast Cancer**

In view of the close functional relationship between PALB2 and BRCA2 and the similar phenotypes associated with biallelic mutation carriers, it was conceivable that monoallelic PALB2 mutations may increase the risk of breast cancer. Five different monoallelic PALB2 truncating mutations were soon found in 10 women from a series of 923 cases with a strong family history of breast cancer (19). These five mutations together were estimated to be associated with, on average, a moderate 2.3-fold increased risk on top of the women’s underlying polygenic risk. Therefore, female monoallelic mutation carriers with a strong family history could be at high absolute risk of breast cancer (20). Moreover, as none of the 1,084 controls had a mutation, this risk estimate could be open to question. At the same time, a founder PALB2 mutation, 1592delT, was identified in approximately 1% of all Finnish breast cancers unselected for family history (21). Using a modified segregation analysis fitted under maximum likelihood theory, the 1592delT mutation was estimated to be associated with about a 6-fold increased risk of breast cancer, and the estimated age-specific cumulative risk by age of 70 years for monoallelic carriers was comparable to that for BRCA2 mutation carriers in the same country (22). Another founder PALB2 mutation, 2323C > T, was subsequently identified and found to be present in ~0.5% of unselected French-Canadian women with early-onset breast cancer (23). PALB2 mutations have now been identified in many

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**Table 1. Malignancies associated with germline mutations in PALB2**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Biallelic (Fanconi Anemia)</th>
<th>Monoallelic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma</td>
<td>5 (62.5)</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Wilms tumor</td>
<td>3 (37.5)</td>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>2 (25)</td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Hemangioendothelioma</td>
<td>1 (12.5)</td>
<td></td>
</tr>
</tbody>
</table>

*n = number of malignancies in PALB2-related FA based on findings in eight families described in published reports (8, 17).
## Table 2. Distribution and frequency of published PALB2 mutations with characteristics of 58 PALB2-related breast cancers in the published literature

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
<th>Breast Cancer Type</th>
<th>DNA Mutations</th>
<th>Protein Change</th>
<th>Cases</th>
<th>Controls</th>
<th>Tumor Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Type</td>
</tr>
<tr>
<td>Canada</td>
<td>Foulkes et al. (23)</td>
<td>Familial/early onset</td>
<td>2323C&gt;T</td>
<td>Q775X</td>
<td>2 of 356 (0.5%)</td>
<td>0 of 6,440</td>
<td>IDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medullary</td>
</tr>
<tr>
<td>Canada</td>
<td>Tischkowitz et al. (34)</td>
<td>Familial</td>
<td>229delT</td>
<td>C77fs</td>
<td>1 of 68 (1.5%)</td>
<td></td>
<td>8 IDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 ILC</td>
</tr>
<tr>
<td>China</td>
<td>Cao et al. (47)</td>
<td>Familial</td>
<td>751C&gt;T 1050_51delAAinsTCT</td>
<td>Q251X</td>
<td>2 of 360</td>
<td>0 of 864</td>
<td>IDC</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>IDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ILC</td>
</tr>
<tr>
<td>Finland</td>
<td>Erkko et al. (21)</td>
<td>Familial Unselected</td>
<td>1592delT</td>
<td>L531fs</td>
<td>3 of 360 (0.8%)</td>
<td>18 of 1,918 (0.9%)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 ILC</td>
</tr>
<tr>
<td>Finland</td>
<td>Heikkinen et al. (24)</td>
<td>Familial Unselected</td>
<td>1592delT</td>
<td>L531fs</td>
<td>19 of 947 (2%)</td>
<td>8 of 1,274 (0.6%)</td>
<td>25/33 IDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3/33 ILC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5/33 other</td>
</tr>
<tr>
<td>Italy</td>
<td>Papi et al. (48)</td>
<td>Familial</td>
<td>2257C&gt;T</td>
<td>R753X</td>
<td>1 of 132 (0.75%)</td>
<td>1 of 300 (0.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Poland</td>
<td>Dansonka-Mieszkowska et al. (25)</td>
<td>Familial</td>
<td>c.509_510delGA</td>
<td>R170fs</td>
<td>4 of 648 (0.6%)</td>
<td>1 of 1,310 (0.08%)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IDC</td>
</tr>
<tr>
<td>South Africa</td>
<td>Sluiter et al. (49)</td>
<td>Early onset</td>
<td>697delG</td>
<td>V233fs</td>
<td>1 of 48 (2.1%)</td>
<td></td>
<td>IDC</td>
</tr>
<tr>
<td>Spain</td>
<td>Garcia et al. (33)</td>
<td>Familial</td>
<td>1056_1057delGA</td>
<td>K353fs</td>
<td>1 of 95 (1.05%)</td>
<td></td>
<td>IDC</td>
</tr>
<tr>
<td>UK</td>
<td>Rahman et al. (19)</td>
<td>Familial</td>
<td>2386G&gt;T 2982insT 3113G&gt;A 3116delA 3549C&gt;G</td>
<td>G796X A95fs W1038X N1039fs Y1183X</td>
<td>1 of 923 0 of 923 0 of 923 0 of 923</td>
<td>0 of 1,084 0 of 1,084 0 of 1,084 0 of 1,084</td>
<td>10 of 923 (1.1%)</td>
</tr>
</tbody>
</table>

Abbreviations: IDC, infiltrating ductal cancer; ILC infiltrating lobular cancer; NS, not stated.
countries (Table 2), with frequencies varying from 0.6 to 2.7% in familial breast cancer cases. However, penetrance estimation from multiple-case families is problematic (19), and due to the limited number of unselected cases studied to date, the average penetrance of PALB2 mutations as a whole, let alone those of specific mutations and/or mutations in different settings (e.g., women with a family history, or with specific risk factors), is not known with certainty.

To date, information for 58 breast cancers arising in PALB2 mutation carriers has been published (Table 2), with about two thirds of these cases emanating from two separate studies in Finland (21, 24). The cancers are frequently high-grade infiltrating ductal type with 40% overall (20 out of 50) having an estrogen receptor (ER)–, progesterone receptor (PR)–, human epidermal growth factor receptor 2 (HER2)– (triple-negative) phenotype. This phenotype does not seem to be mutation specific because, even with the exclusion of the Finnish mutation breast cancers, 8 out of 21 (38%) of the remaining cases are triple negative. In the Finnish study, 7 out of 12 triple-negative breast cancers had a basal-like phenotype (24), and at least two other studies have reported medullary breast cancers (23, 25). It therefore seems that PALB2-related breast cancers might represent a separate category from BRCA1- and BRCA2-related tumors with some overrepresentation of triple-negative tumors, more akin to BRCA1- than BRCA2-related tumors. It is tempting to speculate that this aspect of the phenotype could be related to the nature of the interaction and/or certain functional similarities between PALB2 and BRCA1 (11, 12), or a direct transcriptional activation of the estrogen receptor by PALB2, as has been shown for BRCA1 (26). However, larger numbers of PALB2-related tumors will need to be studied before any firm conclusions can be drawn.

Predisposition to Other Cancers

In addition to breast cancer, BRCA2 mutation carriers are at increased risk of ovarian, pancreatic, prostate cancers, and melanoma, which raises the possibility that PALB2 mutation carriers might also be at increased risk of developing these cancers. Using exonic sequencing, Jones and colleagues identified a germline PALB2 mutation in a familial pancreatic cancer, and when they sequenced PALB2 in 96 other highly selected pancreatic cancer families, a further three mutations were identified (27). A subsequent study of 254 less highly selected families from Canada identified one large deletion mutation (28). Four of the above five PALB2-related pancreatic cancer families included at least one case of breast cancer, and in two families mutations were found in women with both breast and pancreatic cancer. Recently, another study found truncating mutations in 3 out of 81 (3.7%) European pancreatic cancer families, which all included breast cancers (29). Given the rarity of these mutations, PALB2 mutation screening may be of limited help for most pancreatic cancer families, but it should be considered if there is an associated history of breast cancer. Only one prostate cancer family with a PALB2 mutation (the 1592delT Finnish founder) segregating with disease has been reported (21), but a larger study of 178 familial and 285 unselected Finnish prostate cancer families, which all included breast cancer (29). Given the rarity of these mutations, PALB2 mutations, but not BRCA2 mutations, confer...
susceptibility to triple-negative tumors, indicating that defective HR may increase the risk of breast cancer but not necessarily triple-negative disease. Identification of potential new functions shared by BRCA1 and PALB2, but not BRCA2, thus holds promise to uncover the molecular genesis of triple-negative breast cancer. BRCA1 has been implicated in transcriptional regulation of many genes and in cellular redox regulation (37, 38), so it would be interesting to determine if PALB2, a chromatin-associated protein, regulates some of the same genes as BRCA1, and if PALB2 also plays a role in oxidative stress response. Similar to BRCA2/FANCD1 and PALB2/FANCN, mutations in another FA susceptibility gene, BRIP1/FANCJ, are also associated with breast cancer susceptibility (39). BRCA2 and PALB2 are both critical for HR, and BRIP1 also contributes to the process, although the mechanism is unclear. Mutations in yet another critical HR gene, RAD51C, have just been shown to be associated with both FA-like phenotypes and breast and/or ovarian cancers (40, 41). These findings suggest that HR may be what “recombines” cancer and FA. All four proteins act “downstream” of FANCD2/FANCI in ICL repair, which could, in part, explain why mutations in their genes are associated with risk of breast cancer, unlike the other genes encoding FA proteins (42). The reason(s) for differences in the cancer risks and gene groups remain(s) unknown (43). A major impact of BRCA1/BRCA2-related research on cancer intervention has been the recent discovery that BRCA1- or BRCA2-deficient tumor cells are hypersensitive to PARP inhibitors (44, 45), which results in persistence of unrepaired single-strand breaks in DNA, ultimately leading to replication fork collapse that requires HR to restore. Multiple clinical trials of several different PARP inhibitors have been conducted or are underway, and respectable levels of antitumor activity have already been reported for olaparib (AZD2281; ref. 46). Given the similar function of PALB2 in HR, it is not surprising that we found EUFA3141 (FANCN) cells are also hypersensitive to olaparib. Therefore, it is possible that PARP inhibitors, either as single agents or in conjunction with other drugs, could also have clinical utility in cancer patients with germline PALB2 mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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