PALB2/FANCN: Recombining Cancer and Fanconi Anemia

Marc Tischkowitz¹,² and Bing Xia³

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.

Introduction

Genome instability is a hallmark of most cancers, as well as a number of cancer susceptibility syndromes including the recessive childhood disease Fanconi anemia (FA). The clinical phenotypes of FA include diverse developmental defects, progressive aplastic anemia, and heightened susceptibility to cancer (1). Although FA is genetically heterogeneous, consisting of at least 13 complementation groups (FA-A, B, C, D1, D2, E, F, G, I, J, L, M, N, and possibly RAD51C), a unifying feature of all FA subtypes is a cellular sensitivity to DNA interstrand crosslinkers (ICL) such as mitomycin C (MMC) and diepoxybutane, indicating that a DNA repair defect is key to the development of FA (1, 2). Interestingly, the gene underlying the D1 subtype of FA, FANCD1, was found to be BRCA2 (3). Germline, monoallelic (heterozygous) mutations in BRCA2 are associated with an increased risk of breast, ovarian, pancreatic, and prostate cancers, whereas biallelic mutations in the gene lead to FA-D1. As the most important function of BRCA2, thus far established, is to enable homologous recombination (HR) through its control of the localization and function of recombination enzyme RAD51 (4), these discoveries suggest that the recombination function may be a cause of the associations between risks of FA and the above cancers. This notion is supported by the emerging consensus that HR is essential for the completion of ICL repair (5), a common defect of all FA cells.

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 PALB2 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

| Table 1. Malignancies associated with germline mutations in PALB2 |
|----------------------------------------|---------------------------|
| **Tumor Type** | **No. (%)** | **No. (%)** |
| Medulloblastoma | 5 (62.5) | Breast cancer |
| Wilms tumor | 3 (37.5) | Pancreatic cancer |
| Acute myeloid leukemia | 2 (25) | |
| Neuroblastoma | 1 (12.5) | |
| Hemangioendothelioma | 1 (12.5) | |

*n = number of malignancies in PALB2-related FA based on findings in eight families described in published reports (8, 17).
<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
<th>Breast Cancer Cases</th>
<th>DNA Mutations</th>
<th>Protein Change</th>
<th>Cases</th>
<th>Controls</th>
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<th>Loss of Heterozygosity</th>
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<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 2/3</td>
<td>ER+PR+HER2−</td>
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<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>8 ID 2/3 n = 5</td>
<td>6 ER+PR+</td>
<td>No</td>
</tr>
<tr>
<td>China</td>
<td>Cao et al. (47)</td>
<td>Familial</td>
<td>751C&gt;T 1050_51delAAinsTCT</td>
<td>Q251X K353fs</td>
<td>2 of 360 (0.8%)</td>
<td>0 of 864</td>
<td>IDC 2/3</td>
<td>ER+PR+HER2−</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 113 (2.7%)</td>
<td>6 of 2,501 (0.2%)</td>
<td>IDC 2/3</td>
<td>ER+PR+HER2−</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>2 of 1,079 (0.19%)</td>
<td>25/33 IDC 1/3 2/3 n = 3</td>
<td>1 ER+PR+HER2−</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 NS</td>
<td>12/22 ER+PR+HER2−</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>IDC 2/3</td>
<td>ER+PR+HER2−</td>
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<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>5/33 other 3/3 n = 17</td>
<td>IDC NS</td>
<td>ER+PR+HER2−</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 3/3</td>
<td>ER−PR−HER2−</td>
</tr>
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<td>UK</td>
<td>Rahman et al. (19)</td>
<td>Familial</td>
<td>2386G&gt;T</td>
<td>G796X</td>
<td>1 of 923</td>
<td>0 of 1,084</td>
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<td></td>
<td>2982insT</td>
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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 exomic 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 did not find an association with this mutation (30). A study of 95 prostate cancer families, in which the median age of diagnosis in the whole cohort was 49 years, did not identify any pathogenic PALB2 variants (31). PALB2 has not been implicated as a predisposition gene in male breast cancer (24).

To date, there have only been two reports on the occurrence of PALB2 germline mutations in ovarian cancer (26, 32). In the first study, DNA samples of unselected Finnish ovarian cancer cases were screened for the presence of the PALB2 c.1592delT founder mutation. Three out of 593 (0.5%) cancer cases were heterozygous for the founder mutation, compared with a frequency of 0.2% in the controls. The second study identified a mutation in 2 out of 339 unselected Polish ovarian cancers (0.6%), compared with a control frequency of 1 in 1,310 (0.08%; ref. 25). However, one of these cases was subsequently found to also have a BRCA2 mutation.

Putative Genetic Mechanisms of PALB2-Related Carcinogenesis

It is unclear how PALB2 heterozygous mutations might cause cancers. Loss of heterozygosity in PALB2 breast tumors has been shown in only one case (33) out of the nine analyzed cases reported to date, so this does not seem to be a common feature of PALB2 tumorigenesis. In addition, preliminary functional studies have not shown a dominant-negative effect of truncated PALB2 proteins in HR (21, 34), although they may be dominant negative in other as yet unknown functions. It is therefore possible that the second PALB2 allele is somatically mutated in the tumors, as was observed in a PALB2-related pancreas cancer (27). In a similar vein, a heterozygous somatic PALB2 mutation was found in a genomic sequencing study of a metastatic, lobular, ER-breast cancer (35). Another possibility is that the wild-type allele could be epigenetically silenced, although to date no reports have been published on PALB2 promoter methylation in tumors from PALB2 heterozygotes. In contrast, methylation in both unselected and familial tumors has been observed at a 1,512-bp CpG island located in the promoter and exon 1 (36). Specifically, this study found methylation in 4 out of 60 (7%) breast cancers, 4 out of 53 (7.5%) ovarian tumors, and 2 out of 8 (25%) breast cancers arising in BRCA2 mutation carriers. Finally, array CGH studies of PALB2-related tumors have shown differences compared with BRCA2-related tumors (34), with a consistent loss of chromosome 18q. However, the number of PALB2-related tumors was small, and these observations need to be verified by larger studies.

Summary and Perspectives

It is now clear that PALB2 physically and functionally links BRCA1 and BRCA2 to form a “BRCA complex” whose integrity is essential for the avoidance of cancer and FA. Though all three proteins are critical for HR, BRCA1 and to a lesser extent PALB2 mutations, 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.

Acknowledgments

We thank members of the PALB2 Interest Group, particularly William Foulkes, John Hopper, and Robert Winpist, for their very helpful comments. We also thank the Jodi Taiger Lazarus Fund for Breast Cancer Research for contributing toward the publication costs of this article.

Grant Support

Marc Tischkowitz is supported by the Susan G. Komen Foundation for the Cure, the Jewish General Hospital Weekend to End Breast Cancer, and the Quebec Ministry of Economic Development, Innovation and Export Trade. Marc Tischkowitz holds a Fonds de la Recherche en Santé du Québec clinician-scientist award. Bing Xia is supported by the National Cancer Institute (R01CA138804), American Cancer Society (RSG-TBG-119822), and The Cancer Institute of New Jersey.

Received 03/26/2010; revised 07/12/2010; accepted 07/26/2010; published OnlineFirst 09/21/2010.

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Cancer Res 2010;70:7353-7359. Published OnlineFirst September 21, 2010.