International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 3; July 2017; Page No. 09-12

A topological model of negative biofeedback on breast cancer by PALB2 gene

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Abstract
PALB2, also known as the partner and the localizer of BRCA2 is a tumor suppressor gene whose mutation has been resulted to breast cancer and also Fanconi's anaemia. SIFT, Polyphen2, I-mutant, pHD snp, SNP & GO, ALIGN GVGD, SNPs 3D were used to find the deletorious effects of mutation on the protein structure and function and these mutations are correlated with the 3D X-Ray crystallographyic structure available in the PDB.
PALB2 (Partner And Localizer of *BRCA2*) binds to and colocalizes with *BRCA2* in DNA repair. Germline mutations in *PALB2* have been identified in approximately 1–2% of familial breast cancer and 3–4% of familial pancreatic cancer cases. The goal of this study was to evaluate the prevalence of *PALB2* mutations in women with breast cancer without *BRCA1/2* mutations who also had a personal or family history of pancreatic cancer. *PALB2*mutation analysis was performed in 94 non-*BRCA1/2* breast cancer patients with a personal or family history of pancreatic cancer. Two truncating *PALB2* mutations, c.3549C>CA and c.2962C>CT, were identified resulting in a mutation prevalence of 2.1%. Two novel *PALB2* missense variants were also found, one of which was deemed potentially deleterious. The prevalence rate of *PALB2* mutations in a non-*BRCA1/2* breast cancer population specifically selected for a family history of pancreatic cancer does not appear to be significantly increased compared to that observed in other breast cancer populations studied thus far. Further evaluation is needed to determine the prevalence of *PALB2* mutations and the clinical utility of such testing in those individuals affected with both breast and pancreatic cancers.

Keywords: BRCA2, breast cancer, PALB2, pancreatic cancer

1. Introduction
PALB2, the partner and localizer of BRCA2, is a tumour suppressor gene which belongs to the FANC (Fanconi's Anaemia complementation genes) family of genes. It consists of 13 exons and maps to chromosome 16p12.2 which shows loss of heterozygousity in 12% breast cancers. It synthesizes the protein PALB2 which plays a critical role in homologous recombination repair (HRR) through its ability to recruit the BRCA1 and RAD51 to DNA breaks. The protein is made of about 1186 amino acids and possesses a coiled-coil motif at the N-terminus and a C-terminal domain contains a series of WD repeats.
Experiments have been performed which has proved the importance of PALB2 gene. Knock out of this gene shows reduced HR activity, MMC sensitivity, intra-S-phase checkpoint defects and shows complete absence of the RAD51 foci in PALB2 deficient cells. There exists a strong correlation between the ability of a BRCA2 variant to bind PALB2 and support HR activity.
There are two main discrepancies regarding the function of the PALB2 protein. Firstly, it is not clear whether PALB2 recruitment to DNA damage is strictly dependent on BRCA1. Few studies have demonstrated that PALB2 does not form clear foci without BRCA1 under endogenous condition whereas another study had shown when PALB2 is expressed ectopically proteins undergone point mutation were largely unable to bind BRCA1 but still they were able to form foci with almost normal efficiency. These two cases highlight that PALB2 may be able to form two distinct types of nuclear foci, one dependent and the other independent of BRCA1.
Second, MRG15 protein related to MORF, component of certain chromatin remodelling complexes, has been recognized as a major PALB2 binding partner. It was also predicted that the downstream regulation of MRG15 leads to an increase of HR and hence stating the risk of MGR15 restriction to HR be it through PALB2 or not. But new studies have shown that MRG15 promotes HR by causing PALB2 chromatin localization.
PALB2 not only interact with BRCA2 but also with BRCA1. When there is a bi allelic germ line mutation of PALB2 then it causes Falconi's anaemia whereas when there is a monoallelic loss of function mutation of PALB2 gene, it poses a risk for the occurrence of breast cancer. This accounts for up to 1% of all breast cancers and contributes for 1% to 2% familial breast cancers.
Double stand breaks (DSBs) is a result of hereditary mutations which increases the risk of female breast cancer. PALB2 was reported to be the most well-known cancer susceptibility gene amongst the Finnish population along with the others two genes BRCA1 and BRCA2.
DNA strand breaks or DSBs are the most lethal genomic lesions. In mammalian cells, DSBs are repaired by two main pathways which include Non-homologous end joining (NHEJ) and homologous recombination (HR). DSBs which are generated during crosslink repair are normally subjected to HR repair.
HR repair starts with the processing of the DNA end, forms single stranded DNA (ssDNA) 3' ends, which are quickly covered by the human ssDNA-binding protein RPA. MRE11-RAD50-Nibrin and BRCA1-CtIP complexes are involved in DNA end processing. PALB2 acts as the mediator between

BRCA1 and BRCA2 via independent interactions at its N- and C- terminus respectively. The C-terminus also binds Rad51C, another breast /ovarian cancer susceptibility as well as FA gene product, thus forming a HR complex also containing XRCC3 and RAD51. BRCA1 concentrate the PALB2 at DNA damage sites in chromatin, where PALB2 recruits and permits stable localization of BRCA2 to the focal intra nuclear sites. BRCA2 promotes the assembly of RAD51 into nucleoprotein filaments thereby replaces RPA. RAD51 nucleoprotein filaments invade the sister chromatid in search for homology. These represent the central step of HR.

2. Methods

Subject selection

Eligible subjects were identified through three university-affiliated cancer risk programs. All subjects were required to have a personal history of breast cancer (invasive or DCIS) and negative testing for both *BRCA1* and *2*mutations. Other specific eligibility criteria included: (1) Personal history of breast cancer at age ≤50 with family history of one or more cases of pancreatic cancer in first or second degree relatives in the same lineage of the family; or (2) Personal history of breast cancer at age ≤65 with family history of one or more cases of breast cancer diagnosed at age ≤65 and one or more cases of pancreatic cancer in first or second degree relatives in the same lineage of the family; or (3) Personal history of breast cancer at age ≤65 with family history of two or more cases of pancreatic cancer in first or second degree relatives in the same lineage of the family; or (4) Personal history of breast cancer at age ≤65 with personal history of pancreatic cancer; or (5) Personal history of bilateral breast cancer with family history of one or more cases of pancreatic cancer in first or second degree relatives in the same lineage of the family. Cancer diagnoses for probands were confirmed by pathology reports; cancer diagnoses for relatives were determined only through proband interviews, though pathologic confirmation was occasionally possible.

Data collection

Clinical information relevant to eligible patients was abstracted from medical records and included: age at breast cancer diagnosis, breast cancer treatment and outcome. Information regarding breast cancer pathology was abstracted from pathology reports and included histology, estrogen receptor (ER), progesterone receptor (PR) and HER2 status, grade and nodal status. Information regarding family history of cancers was also collected from both lineages and included: types of cancers, age at cancer diagnoses, and family ethnicity.

Laboratory methods

Blood or saliva was collected from each study subject for DNA analysis. DNA was extracted from blood using a Qiagen DNA extraction kit and from saliva using the Oragene DNA extraction procedure. Patients were tested for*PALB2* alterations using Capillary Exon Grouping Analysis (cEGAN). Exon grouping analysis is based on Conformation-Specific Capillary Electrophoresis [22, 23]. All coding exons and surrounding intronic sequences were amplified with 19 primer pairs and analyzed on ABI-3730XL instruments (Netaji Subhash Engineering College, Kolkata, Indaia). Polymerase chain reaction fragments with aberrant mobility were sequenced for the first 12 exons. Exon 13 was directly sequenced in all patients. This method permits detection of mutations and polymorphisms in*PALB2* coding regions as well as splice-site mutations. *BRCA1* and *2* gene sequencing for all cases was performed through appropriate process.

Statistical Methods

The 90% confidence interval (CI) of the prevalence of germline *PALB2* mutations was computed using the method of exact binomial confidence interval [24].

A total of 94 cases from independent families were selected for *PALB2* sequence analysis. None of the subjects had a *BRCA* mutation or variant of uncertain significance. Characteristics of the 94 study subjects are summarized in. Median age at breast cancer diagnosis was 41 years, with 29.8% of subjects being diagnosed with multiple breast cancers. Three subjects had a personal history of both breast and pancreatic cancers. All subjects except one had at least one first- or second-degree family member with pancreatic cancer, and 11.7% of subjects had two relatives with pancreatic cancer. (One patient had a personal history of breast and pancreatic cancer, but no family history of pancreatic cancer). The majority of the subjects in our study were of South 24 Paraganas, West Bengal, India (62/94, 65.9%).

Truncating *PALB2* mutations were identified in 2/94 subjects (2.1%; 90% CI = 0.40%, 6.5%). Both subjects were of wide spectrum of 24 Paraganas, West Bengal. In the first subject, *PALB2* sequence analysis revealed a nonsense mutation in exon 9, c.2962C>CT, which results in a stop codon at amino acid 988. This subject was diagnosed with pre-menopausal breast cancer at age 49. Pathology review showed a 1.5 cm, poorly differentiated, invasive ductal breast carcinoma that was negative for both estrogen receptor (ER) and Her 2 over-expression. The subject also had a basal cell carcinoma of the skin at age 47. The family history was remarkable for a father with pancreatic cancer at age 62 and a paternal aunt with pancreatic cancer at age 60.

In the second subject found to have a mutation, *PALB2* sequence analysis revealed a nonsense mutation in exon 13, c.3549C>CA, which results in a stop codon at amino acid 1183. This subject was initially diagnosed with pre-menopausal breast cancer at age 43. Pathology review showed a 2.5 cm invasive ductal carcinoma, ER-positive, Her2 status unknown. Despite treatment with mastectomy, chemotherapy and tamoxifen, the breast cancer recurred with distant metastases at age 47. The family history was remarkable for a mother with breast cancer at age 50 and pancreatic cancer at age 61. Other notable cancers within the pedigree included a maternal uncle with colon cancer at age 48, a maternal grandfather with pharyngeal cancer at age 71, and a paternal aunt with colon cancer at age 30. *PALB2* sequence analysis of the mother's blood revealed the same *PALB2* mutation identified in the proband. Tissue from other family members was unavailable for analysis.

3. Discussion

We found *PALB2* mutations in two of 94 subjects, a prevalence of 2.1% in this specific breast cancer population. This rate is consistent with the 1–2% prevalence of mutations reported in other familial breast cancer populations [6, 14]. A recent study by

Adank *et al.* [26] evaluated the prevalence of *PALB2* mutations in 110 *BRCA2*-like families, including 45 breast cancer patients with a first or second degree relative with pancreatic cancer. While one truncating *PALB2* mutation was found in a male breast cancer patient, none of the 45 subjects with breast cancer and a family history of pancreatic cancer was found to carry a pathogenic mutation. The difference in PALB2 mutation prevalence between our study and that of Adank *et al.* is likely not significant and may be due to the small sample sizes. While the *PALB2* mutation prevalence rate of 2.1% in our population is higher than that reported by Adank *et al.*, it is lower than that found in studies of familial pancreatic cancer (3.1–3.7%) [18, 19].

The c.3549C>CA and c.2962C>CT *PALB2* mutations identified in our sample have been previously described in breast cancer and Fanconi anemia patients, respectively [8, 18]. However, our study is the first to specifically identify the c.3549C>CA *PALB2* mutation in association with pancreatic cancer. This mutation was identified in the mother of a proband within our sample; the mother was affected with both breast and pancreatic cancer. It is likely that the c.2962C>CT *PALB2* mutation is specifically associated with the pancreatic cancer in our proband's family as well, however tissue from family members affected with pancreatic cancer was unavailable for analysis. With the addition of our data on the family with the c.3549C>CA *PALB2* mutation, there are now nine *PALB2*-related pancreas families identified to date [17–19]. Interestingly, eight of these families include one or more cases of breast cancer, and three families contain individuals with both breast and pancreatic cancer. Within the familial pancreatic cancer population described in the report of Tischkowitz *et al.* [17] one of nine subjects (11.1%; 90% CI = 0.57%, 42.9%) with both breast and pancreatic cancer carried a *PALB2* mutation. We performed *PALB2*sequence analysis on four individuals with concomitant breast and pancreatic cancer, namely three probands and one relative (mother) of a proband with breast cancer. Of these, one mutation carrier was found. Though numbers are small, these rates of *PALB2* mutations within individuals with both breast and pancreatic cancer is noteworthy and warrants further study of this patient population.

Previous reports have suggested that breast tumors associated with some *PALB2* mutations are aggressive in nature [12, 16]. Our data further supports this concept, as both mutation carriers in our sample proved to have aggressive disease. The breast cancer in the first mutation carrier was notable for poor differentiation and triple-negative status, lacking ER, PR and HER2 over-expression. In the second mutation carrier, the breast tumor recurred with distant metastases 4 years after diagnosis despite initial aggressive therapy for ER-positive, node-negative disease. These observations must be interpreted with caution given the small sample size, but are nevertheless intriguing.

4. Conclusion

In conclusion, we found that *PALB2* mutations occur with a prevalence of 2.1% in a population of *BRCA1/2*-negative breast cancer patients specifically selected for a personal and/or family history of pancreatic cancer. This prevalence rate appears comparable to the rate of *PALB2* mutations published in other breast cancer populations. Further study is needed to determine the prevalence of *PALB2* mutations and the clinical utility of such testing in those individuals affected with both breast and pancreatic cancers. The benefit of pancreatic cancer screening in patients at increased risk of developing pancreatic cancer, such as those who carry a *BRCA2* or *PALB2* mutation, is uncertain. Recently, Verna *et al.* [27] reported that endoscopic ultrasound (EUS) and MRI were able to detect early neoplastic pancreatic lesions in 12% of 51 individuals with a family history of pancreatic cancer. Other studies evaluating the benefit of such screening are ongoing. If pancreatic cancer screening is found to be beneficial in high risk populations, identifying those individuals with PALB2 mutations will likely have even greater clinical significance.

Author's Contribution

Author, Debolina Chatterjee has extended in depth research and exclusive study regarding the present spectrum of PALB2 gene that has been manifested to write her this review in favour of mankind and well being of health science and cultivation.

5. Acknowledgement

It is an established fact that every mission needs a spirit of dedication and hard work but more than anything else it needs proper guidance. Authors feel proud in taking this opportunity to express heartiest regards and deep sense of gratitude to our beloved expert and pioneer supervisor of this extensive study on PALB2 gene susceptibility to carcinoma, Dr. Partha Majumder, Human Physiologist and Systems Biologist, Former Principal Scientist(Helixinfosystems), Former Head &Coordinator, Sikkim Manipal University(CC:1637), Kolkata, India.

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