Functional evaluation of BRCA1 variants located in coiled-coil domain: PALB2 interaction approach

Elias, A. B. R.[1], De Gregoriis, G.[1], Suarez-Kurtz, G.[1], Monteiro, A.N.[2], Carvalho, M.A.[1,3]
[1]Instituto Nacional de Câncer, Rio de Janeiro – Brazil. [2]H. Lee Moffitt Cancer Center, Tampa, FL. - USA
[3]Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Rio de Janeiro - Brazil

INTRODUCTION

Hereditary breast cancer (BC) accounts for 10 to 15% of total BC cases. The majority of hereditary cases can be attributed to alterations in the breast and ovarian cancer predisposing gene (BRCA1), a crucial gene for the maintenance of genome integrity and cellular processes **(Figure 1)**. Genetic counseling based on comprehensive analysis of alterations helps not only the early identification of individuals in high-risk of developing cancer, but also helps affected individuals to seek guidance and to adopt preventive procedures. However, many of the mutations found in genetic studies cannot be classified neither in high-risk or in non-high-risk alleles because they do not have a clear designation with cancer association. In these cases, the mutations remain as unclassified variants (or variants of unknown significance, VUS). Functional assays may work to circumvent this problem by associating protein biological functions and the integrity of specific domains.

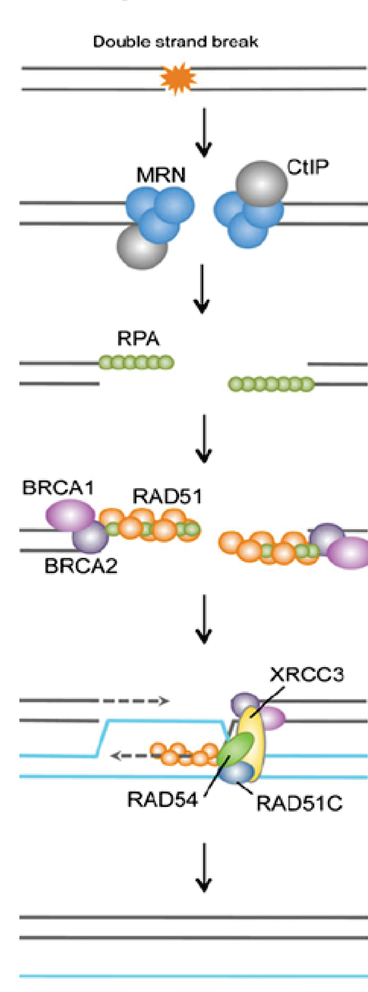

Figure 1: Schemaic reprsentation of double-strand breaks (DBS) repair by HR. The repair of DSB during homologous recombination is initiated by the resection of the DNA ends through the action of the MRN complex and CtIP protein to generate single-stranded DNA. The replication protein A (RPA) bounds at the single-stranded; BRCA1 and BRCA2 are recruted to damaged site and RAD51 invade the homologous template. Next, Holliday junction is generated to prime DNA synthesis and restore genetic information that was disrupted by the DSB (Brochier and Langly, 2013).

MATERIALS AND METHODS

A initial set of BRCA1 missense variants (group 1, 7 varinats) was selected based in Breast Cancer Information Core database. A second set of 14 missense variants (group 2) was selected after an *in silico* prediction. High or moderate-risk cancer association predicted by the Align GVGD algorithm and the predictive position importance to PALB2 interaction were used as inclusion criteria **(Figures 2 e 3)**. BRCA1 variants were generated by site-directed mutagenesis for further cloning into pCDNA3 vector **(Figure 4)**. pCDNA3 constructs enclosing the GAL4 DNA binding domain (GAL4DBD) fused to the BRCA1 WT or variants coding sequences (limited by exons 11-24, amino acids 1315-1863, pCDNA3-GAL4DBD:BRCA1) were used in a M2H assay together with a pVP16 construct enclosing the coding sequence of the transcriptional activator VP16 fused to the N-terminal regions of PALB2 (amino acids 1-319, pVP16-PALB2).

BRCA1 (Human) – I L T T Q Q R D T M Q H N L I K L Q Q E M A E L E A V L E Q H G S Q

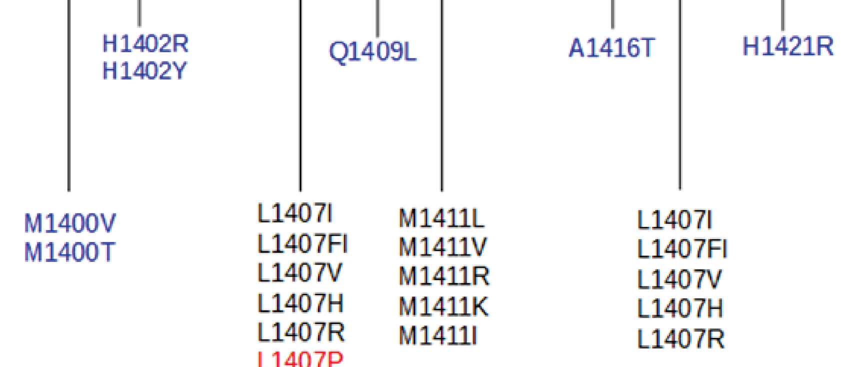

Figure 2: Schemetic representation of BRCA1 variants. BRCA1 coiled-coil region is depicted. Variants from the group 1 are represented in blue and variants from de group 2 are in black. The variant L1407P (in red) is used as a negative control for M2H interaction assay with PALB2.

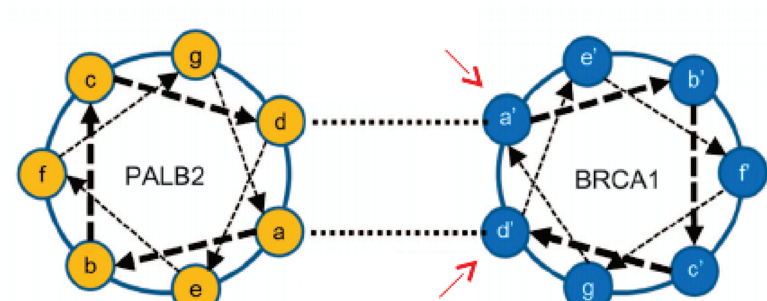

Figure 3: Schemetic of BRCA1 and PALB2 residues interaction. BRCA1 amino acids residues at a' and d' positions in coiled-coil are described as relevant for PALB2 interaction.

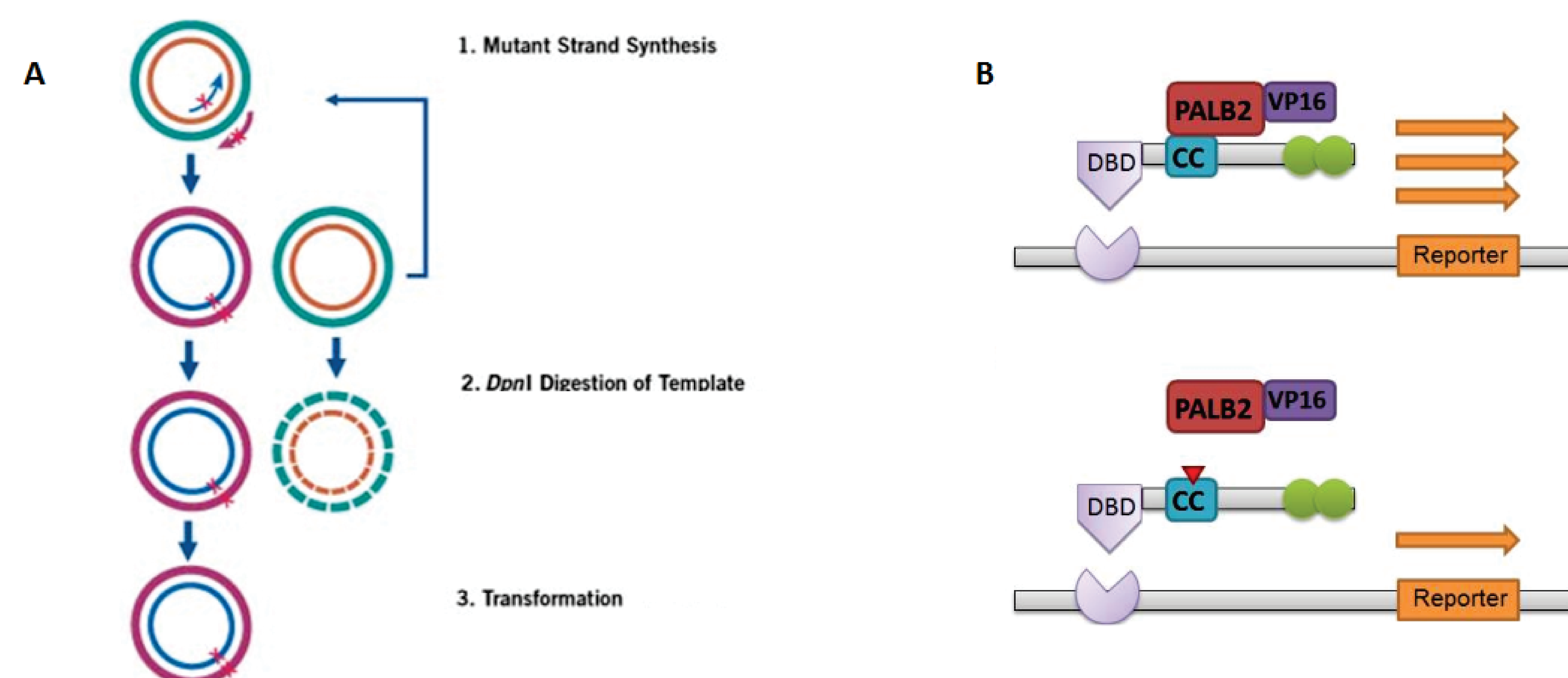

Figure 4: Methods. (A) Site-directed mutagenesis (QuikChange Kit, Agilent Genomics) and **(B)** the mammalian two-hybrid PALB2 interaction assay.

RESULTS

So far, 16 variants (out of 21) were generated and target mutations were confirmed by sequencing; 11 variants (5 variants from group 1 and 6 from group 2) were already evaluated by the M2H assay. All variants evaluated from group 1 showed PALB2 interaction levels similar to BRCA1 WT. M1400V, M1400T, A1416T, Q1409L and H1421R were predicted as variants of small-risk for cancer association (Class C0 – C15), consistent with our experimental data **(Figure 5).**

M1411L and L1418I were also predicted as small-risk variants, although they were predicted to be situated in important positions for PALB2 interaction. Even so, substitutions did not abolish protein interaction, in consonance with algorithms prediction. The 4 remaining variants (L1407R, M1411R, L1418S e L1418V), all predicted to be situated in important positions for PALB2 interactions, presented activity similar to the negative control, suggesting a pathogenic behavior. It is worth of note that all evaluated variants were classified in high-risk cancer association (Class C65), but L1418V that was classified in moderate risk (Class C25). Substitutions abolished protein interaction, as expected **(Figure 6)**

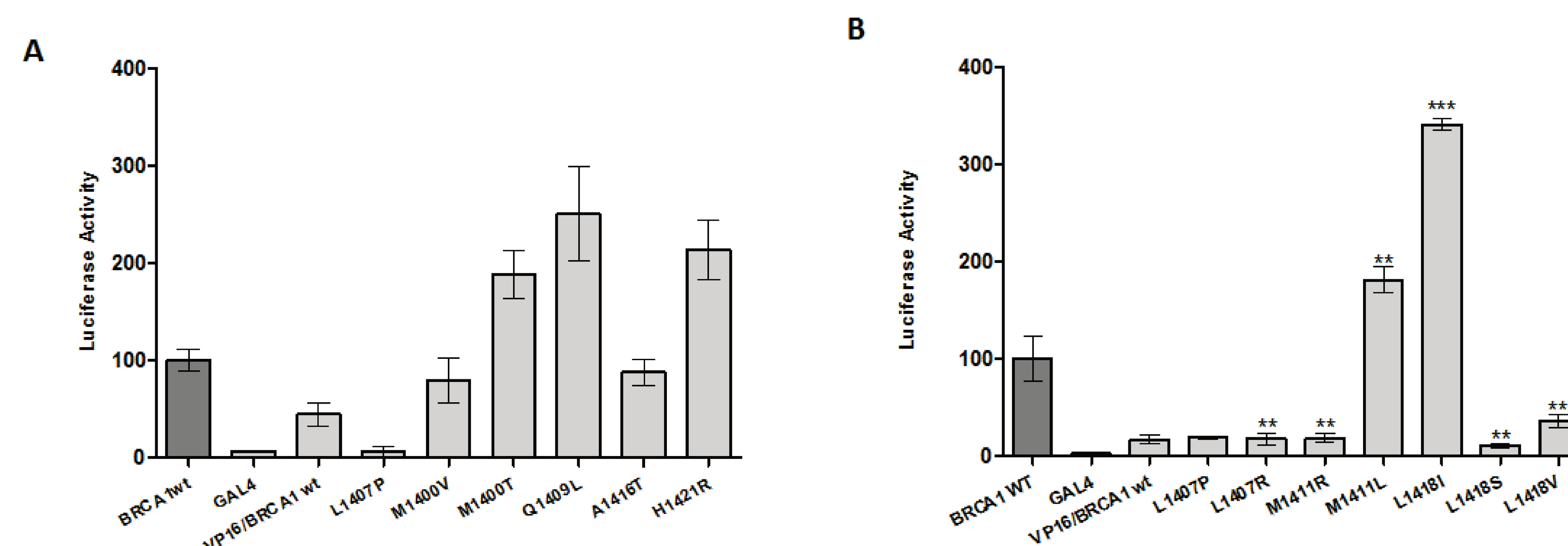

Figure 5: BRCA1 variants ability to Interact with PALB2 (M2H assay). HEK293FT cells were cotransfected with the reports (pG5luc and pGR-TK) and BRCA1 (wt and variants) and PALB2 constructions. The M2H was performed 24h after the transfection. (A) Luciferase activity observed for group 1 variants and (B) for group 2. Data represent activity of two independent experiments, statistical significance was determined by one-way ANOVA and Dunnet's post hoc analysis (mean ± SEM; ***p<0,0001).

CONCLUSION

The interaction of BRCA1 and PALB2 is essential for genomic integrity maintenance; therefore, evaluate the ability of BRCA1 variants to interact with PALB2 may possibly work as a functional assay to predict a pathogenic behavior of missense mutations in BRCA1 coiled-coil region.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

INCA SUS MINISTÉRIO DA SAÚDE BRASIL GOVERNO FEDERAL