Characterization of BRCA1 SQ-cluster phosphorylation status in its interaction with PALB2 in the DNA damage response

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INTRODUCTION

Germline mutations in the *BRCA1* tumor suppressor gene are associated with increased risk of hereditary breast and ovarian cancers. Its encoded protein plays a pivotal role in the maintenance of genomic integrity through homologous recombination (HR)-mediated DNA damage repair. BRCA1 works as a scaffold to promote the formation of different protein complexes participating in several steps of HR pathway. A particular complex, formed by different cancer susceptibility gene products, including PALB2 and BRCA2, plays a crucial role regulating RAD51 recombinase activity, the crucial step of HR pathway. Mechanistically, BRCA1 and PALB2 interact through the heterodimerization of their coiled-coil motifs and mediate the recruitment of BRCA2 and consequently RAD51 to the DNA damaged sites. Recently, the association between BRCA1 and PALB2 was shown to be orchestrate in a cell cycle and DNA damage-dependent manner by post-translational modifications in PALB2, such as ATR and CDKs mediated phosphorylations. Interestingly, ATR and CDKs phosphorylations sites predicted in BRCA1 are linked to the PALB2-interaction region. Those sites are associated with DNA damage response (DDR), but it is not clear whether they modulate BRCA1/PALB2 interaction.

OBJECTIVES

This work aims to evaluate the role of BRCA1 phosphorylation in mediating PALB2 heterodimerization associated to DDR.

METHODS AND RESULTS

BRCA1/PALB2 interaction will be evaluated by mammalian two-hybrid and GST-pulldown assays using phosphomimetic variants generated by site-directed mutagenesis strategies - serine residues will be substituted by glutamic acids or alanine residues (mimicking a phosphorylated or unphosphorylated motif, respectively). The DDR status will be accessed by HR-proficiency assays and sensitivity to ionizing radiation/PARP inhibitors in cells expressing the phosphomimetic BRCA1 variants. Literature curation led to identification of 8 predicted serine residues target by ATR/CDKs in the PALB2-interaction region of BRCA1 protein structure. Plasmidial constructs are been generated and pilot assays testing BRCA1/PALB2 interaction are been performed.

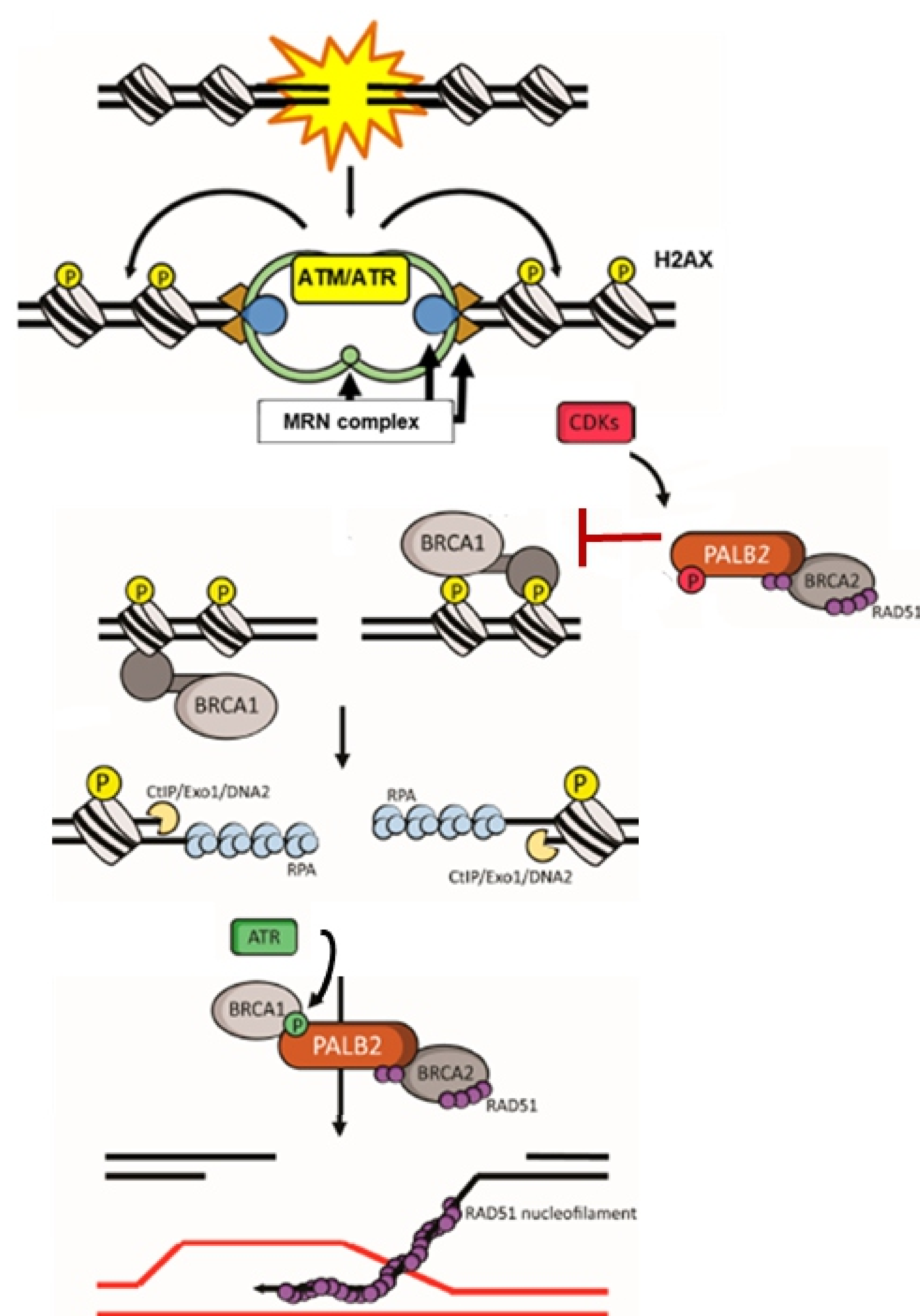

Figure 1: The phospho-modulation of BRCA1/PALB2 association in homologous recombination (HR)-mediated DNA repair. Schematic representation of HR pathway. DNA double strand breaks are recognized by MRN sensor complex (MRE11-RAD50-NBS1) leading to ATM/ATR activation and H2AX phosphorylation (gH2AX), which in turn, amplifies the DNA-damaged signal. This phenomenon works as a platform to downstream events leading to BRCA1 recruitment and 5' DNA end resection process (performed by CtIP, EXO1, DNA2 and MRN complex nucleases activities stimulated by BRCA1). A CDK-mediated PALB2 phosphorylation abrogates its association with BRCA1 at this moment. Afterwards, RPA caps exposed single-strand DNA and are substituted by RAD51 recombinase in a process dependent on BRCA1/PALB2/BRCA2 complex. At this point, a ATR-mediated PALB2 phosphorylation promotes its association with BRCA1, and consequently, RAD51 recruitment and DNA damage resolution.

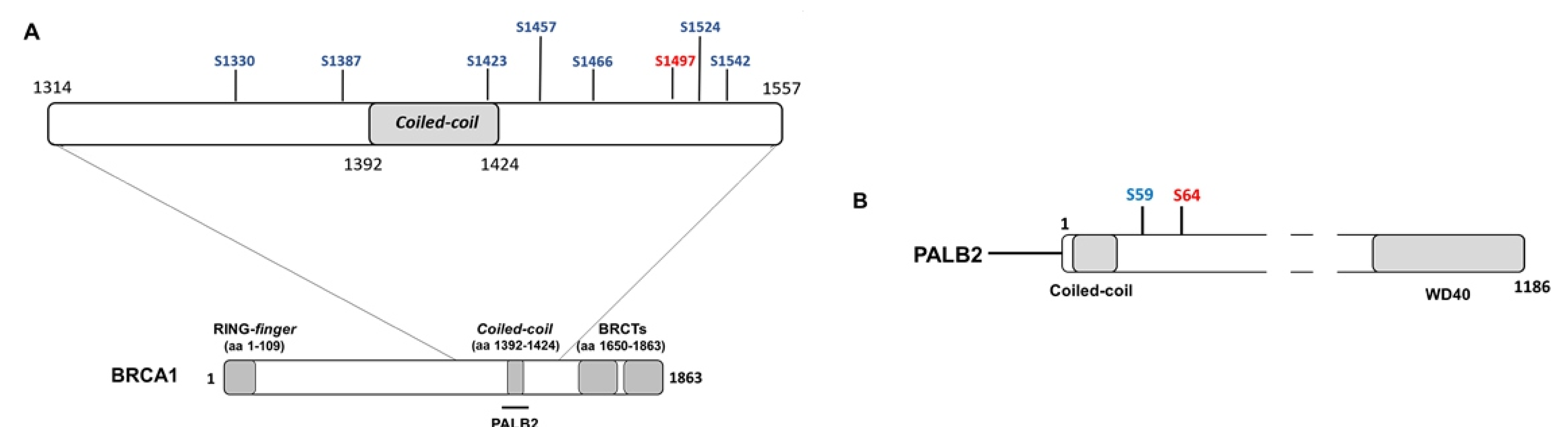

Figure 2: Phospho-modulation of BRCA1/PALB2 association. (A) Schematic representation of BRCA1 protein with the SQ-cluster depicted and **(B)** PALB2 protein. CDKs phosphosites are highlighted in red and ATM/ATR in blue. Protein domains are represented in gray.

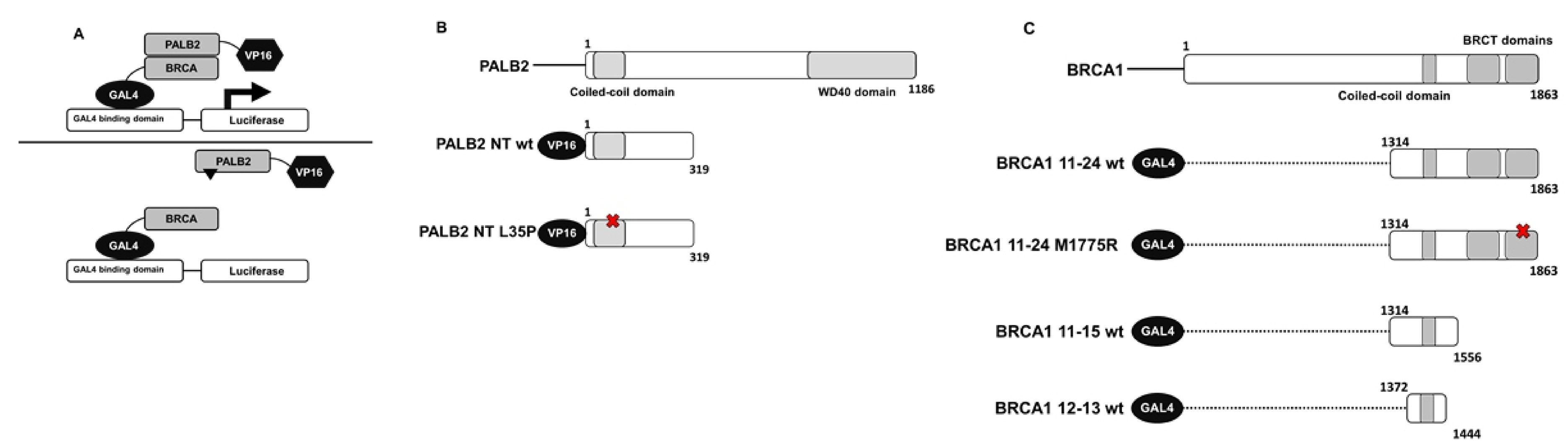

Figure 3: Mammalian two-hybrid (M2H) assay. (A) Schematic representation of M2H assay. **(B)** Schematic representation of PALB2 N-terminal (NT) and (C) BRCA1 constructs.

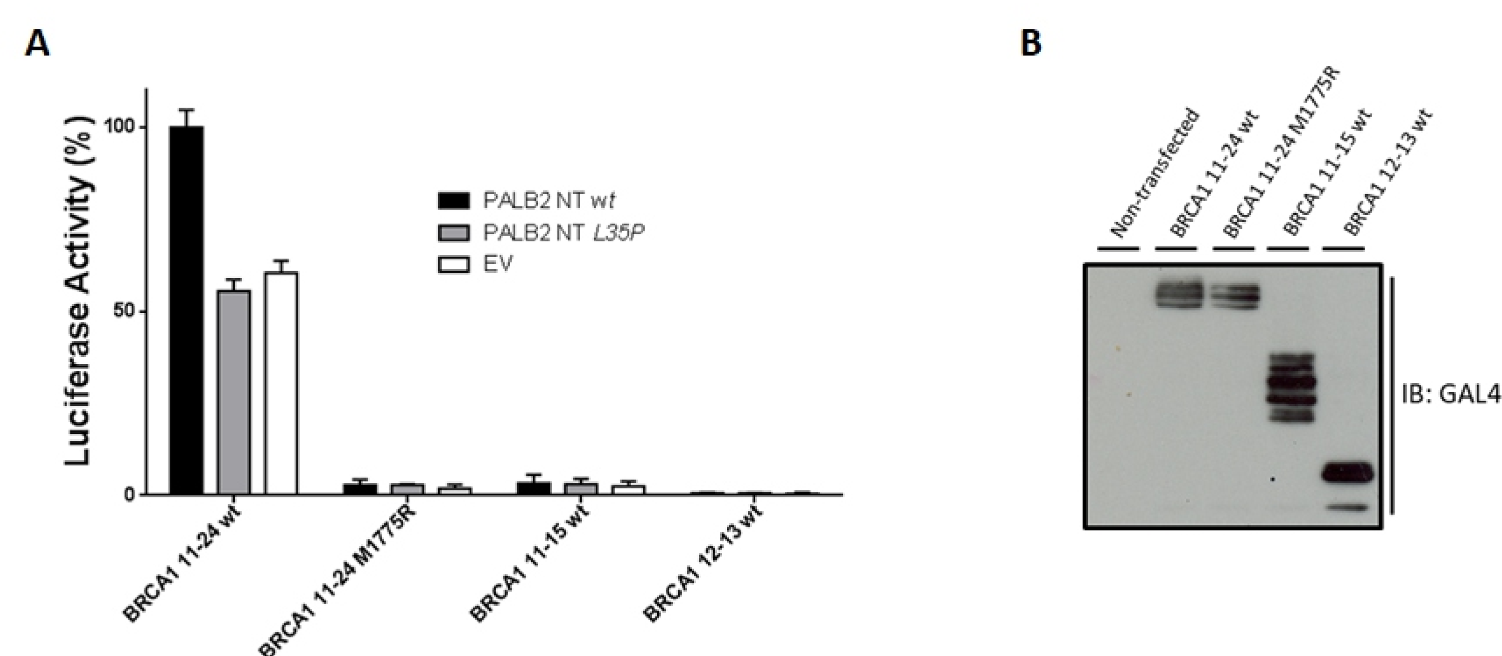

Figure 4: Mammalian two-hybrid assay. (A) HEK293FT cells were cotransfected with the reporters (pG5*luc* and pGR-TK), BRCA1 constructs (BRCA1 11-24 wt, BRCA1 11-24 M1775R, BRCA1 11-15 or BRCA1 12-13) and PALB2 coding plasmids (wt, L35P or empty vector (EV)); the mammalian two-hybrid assay was performed 24h post-transfection. Data represent luciferase activity of a representative experiment of a series of independent experiments. **(B)** Immunoblotting analysis of M2H proteins.

CONCLUSIONS

The comprehension of the molecular mechanisms behind BRCA1/PALB2 association is extremely important for a better understanding of maintenance of genomic integrity and cancer predisposition. It also impacts in patient surveillance and in the development of new treatment based on BRCA's status.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

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