Investigating the Effects of Peptide Mimics on the Binding Interaction between BRCA1 and PALB2

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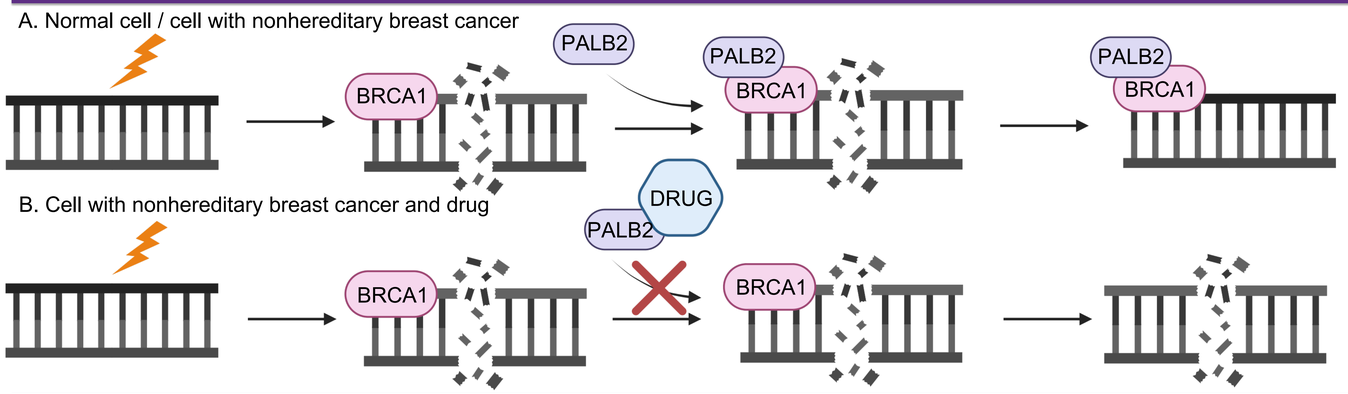
Introduction



Left: When DNA is damaged, PALB2 (purple) is recruited to the site of the double stranded break and forms a heterodimer with BRCA1 (pink). This complex aids in repairing the breaks through homologous recombination. When this complex cannot form due to hereditary BRCA1 mutations, DNA damage repair is halted, leading to an increased risk of breast cancer. In cancer cells without hereditary BRCA1 mutations, DNA is repaired normally, leading to proliferation of the cancer. We hypothesize that small drug molecules can be used to bind PALB2, inducing a BRCA1 loss of function mutation, and preventing the binding of the proteins, thus causing DNA double stranded breaks to remain unresolved, causing the death of cancer cells.

Objectives

- Evaluate the ability of macrocycle molecules on inhibiting the protein-protein interaction between BRCA1 and PALB2.
- Identify the optimal sequences of the peptide mimicking molecule to bind PALB2 with higher affinity than its binding partner, BRCA1.
- Assess the inhibitory effectiveness of stapled peptides targeting the BRCA1-PALB2 interaction and compare their efficacy to the macrocycle molecules.

Macrocycles and Stapled Peptides

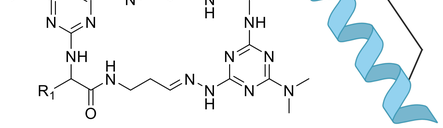
Normal BRCA1 sequence: QRDTMQHNLIKLQQEMAELEAVLEQHGSQPS

Sequence of Peptide 1: Ac-YNLXSLQQEMXAL-NH_2

Sequence of Peptide 2: Ac-TMQXNLESLQXEMA-NH_2

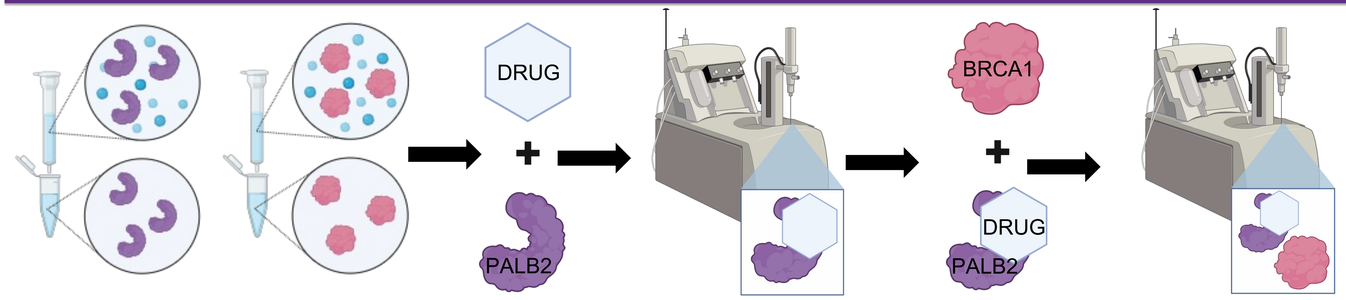
Sequence of Peptide 3: Ac-LQXEMAYLEXVL-NH_2

Above: BRCA1 sequence compared to the sequences of the stapled peptides. The ends of each peptide were acetylated and aminated for ease of purification and to increase solubility. The pink amino acid codes signify matches with the BRCA1 sequence. The X signifies the modified amino acid residue for the hydrocarbon “staple” used to stabilize the alpha helix structure of the stapled peptides.

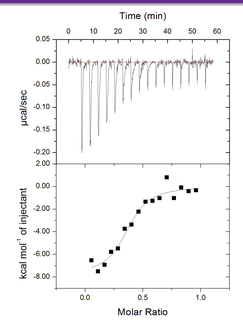


Left: Macrocycles are cyclic, amino acid-like structures. The R1 signifies the placement of the amino acid side chains, such as V, Y, K, and L. Stapled peptides are alpha-helical structures connected by a hydrophobic staple.

Methods

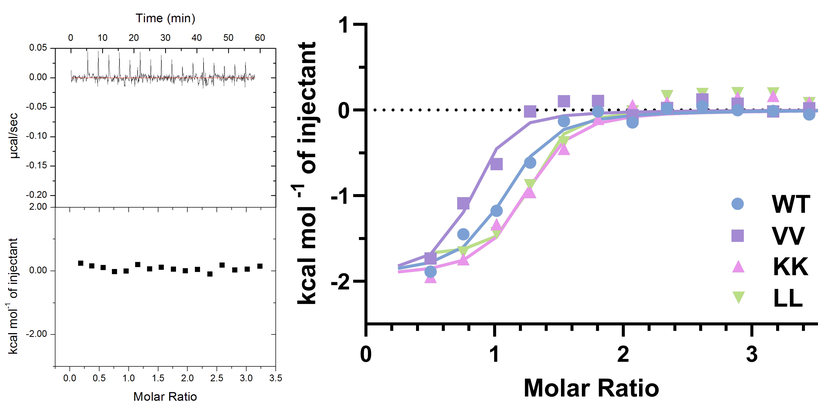


Above: BRCA1 (pink) and PALB2 (purple) are purified from E. Coli cells using charge and size exclusion chromatography. The interaction of PALB2, BRCA1, and the small drug molecule are studied using isothermal titration calorimetry. The small drug molecule is first titrated into PALB2 to visualize drug-PALB2 binding. Next, BRCA1 is titrated into the mixture to assess if the drug prevents the formation of the PALB2-BRCA1 heterodimer.



Normal binding interaction between BRCA1 and PALB2

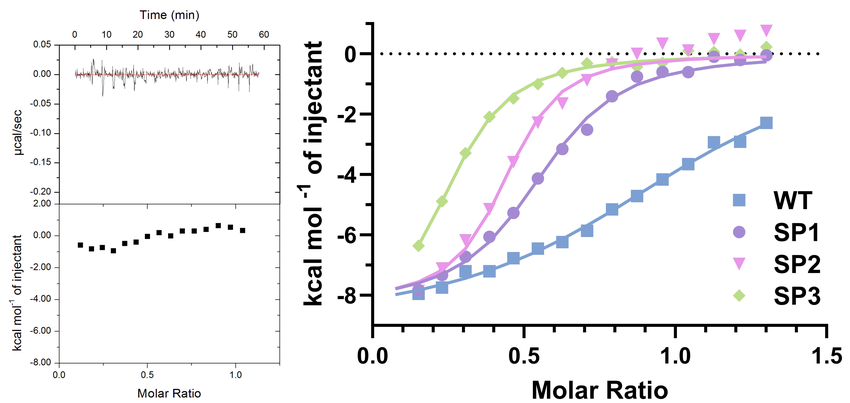
ITC data suggest that macrocycle molecules do not disrupt BRCA1-PALB2 binding interaction



Top left: KK into PALB2 titration shows there is no binding. The top and bottom sections of the graph display that there are no heat changes as seen in the wild type data. All other macrocycles show similar results.

Top right: Results from the wild type BRCA1 titration into PALB2 only (WT) or PALB2 in the presence of VV, KK, or LL macrocycles. The similarity of saturation curves suggest that the macrocycles are not interrupting BRCA1 and PALB2 binding.

ITC data suggest that stapled peptides interact with BRCA1 and PALB2 binding



Top left: SP3 into PALB2 titration shows there is no binding interaction. This could be due to the fact both the peptide and PALB2 are alpha helices, therefore, there may be no heat changes upon binding. All other stapled peptides show similar results.

Top right: Results from the wild type BRCA1 titration into PALB2 only (WT) or PALB2 in the presence of SP1, SP2, and SP3. The movement of the saturation curves to the left suggest that the stapled peptides are competing with BRCA1 for the binding site of PALB2.

Conclusions and Future Directions

- ITC data suggests there is not detectable binding of the macrocycles or stapled peptides with WT PALB2.
- Macrocycle molecules also do not disrupt the BRCA1-PALB2 binding interaction, while stapled peptides may be competing with PALB2 for the binding site of BRCA1.
- Our results highlight the difficulty in interrupting highly conserved protein-protein interactions.
- Utilization of in-cellulo assays could be useful to test the cell-permeability of these drugs.
- It may be worthwhile to test variants of the stapled peptide structures by altering the hydrophobic staple (this could be placement or chemistry) or amino acid sequence.
- Alter the structure of the macrocycle to better match the sequence of BRCA1.
- High throughput screening of stapled peptides and macrocycles may be useful for efficient evaluation of multiple peptide mimics at one time, while requiring fewer resources than ITC.

References and Funding

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