Investigating the effects of patient variants and phosphorylation on the BRCA1/PALB2 interaction

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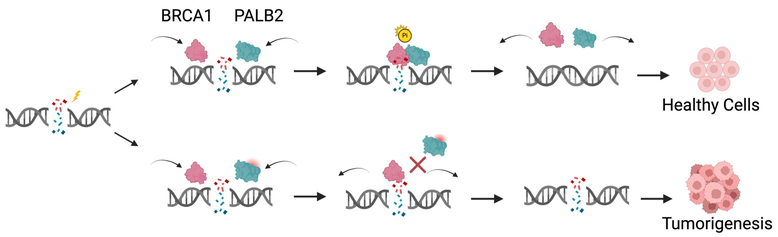
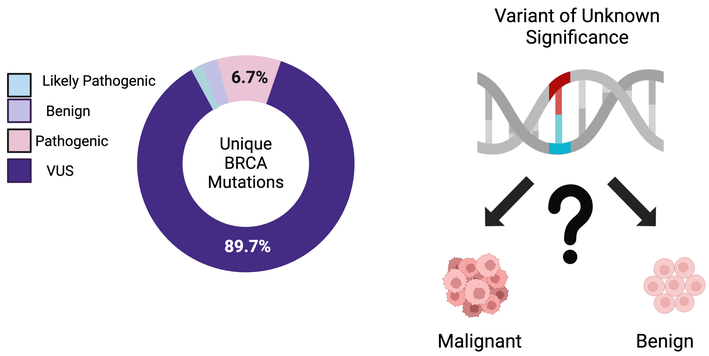
Introduction

BRCA1/PALB2 interaction is crucial for DNA damage repair



Above: In the presence of DNA damage, BRCA1 and PALB2 are phosphorylated and recruited to the damage, where they form a heterodimer resulting in repaired DNA through homologous recombination. In the absence of a successful heterodimer, DNA damage persists and tumorigenesis can occur.

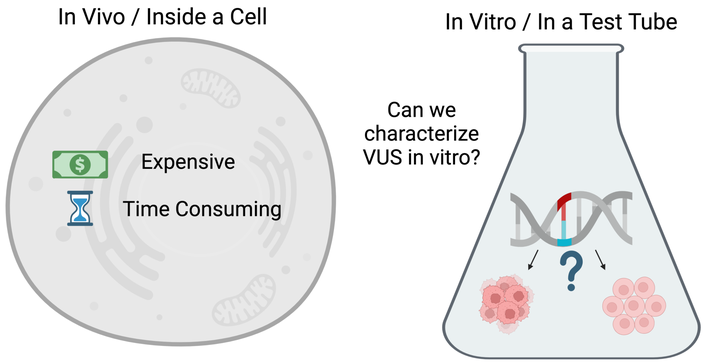
Variant of Unknown Significance



Above: Of the 72,467 documented BRCA mutations, only 10.3% of them have been classified as either benign, likely pathogenic, or pathogenic. There are over 65,000 unclassified variants of unknown significance!

Objective

Create in vitro conditions to test the function of variants that mimic conditions inside the cell. Does phosphorylation need to be accounted for in vitro to assess VUS?

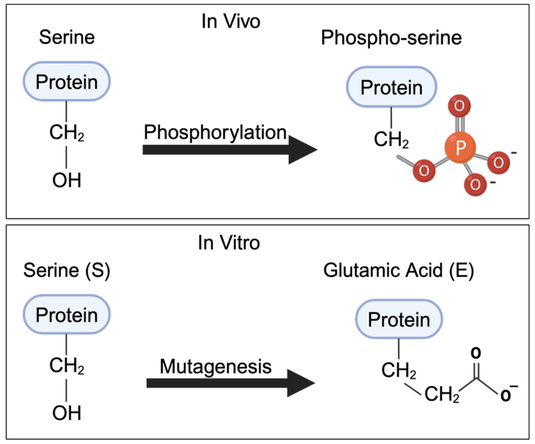


Above: In vivo assays are costly and time consuming compared to in vitro assays.

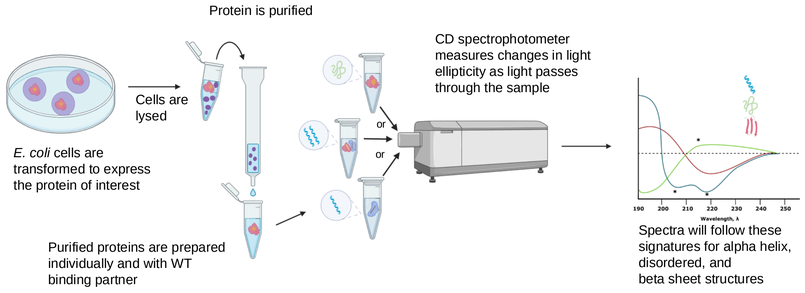
Methods

Site-Directed Mutagenesis

Right: *In vivo* phosphorylation involves the attachment of a phosphate group to serine residues. To mimic this state *in vitro*, site-directed mutagenesis is used to mutate a serine (S) to a glutamic acid (E), providing a negative charge that mimics phosphorylation.

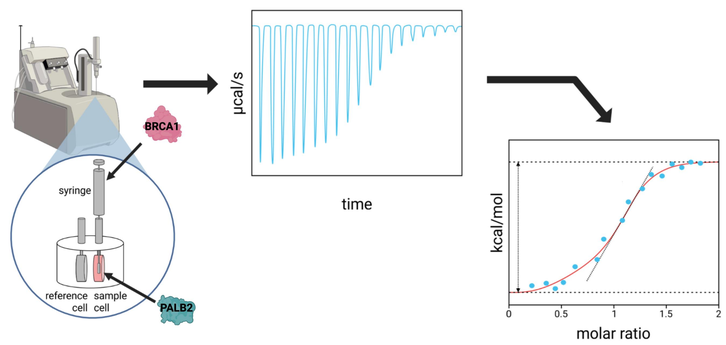


Protein Purification and Circular Dichroism



Above: After transformation and expression of human protein in bacterial cells, protein is lysed from bacterial cells and purified. Then, purified product is analyzed via circular dichroism to determine secondary structure.

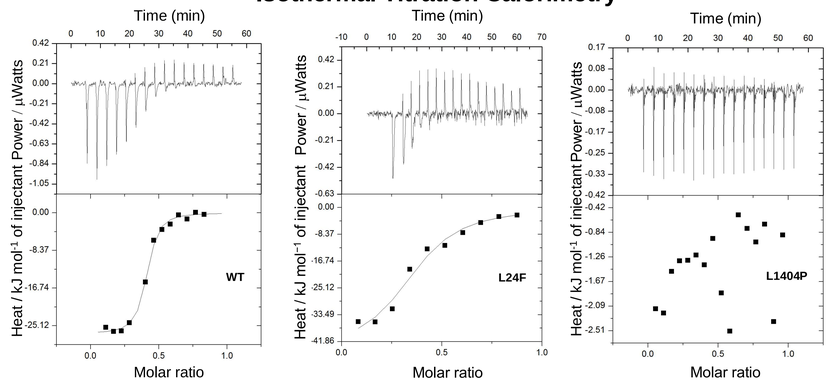
Isothermal Titration Calorimetry



Above: Schematic describing the steps of isothermal titration calorimetry, which measures heat release upon binding throughout the titration of BRCA1 into PALB2.

Results

Isothermal Titration Calorimetry



Above: Isothermal titration calorimetry experiments showing the titration of wild type (WT) BRCA1 into WT PALB2, WT BRCA1 in L24F PALB2, and L1404P into WT PALB2.

Summary of Findings

Protein	Variant	Structure	Heterodimerization
BRCA1	T1394E	?	?
BRCA1	M1400P	?	?
BRCA1	M1400T	?	✗
BRCA1	L1404P	?	✗
BRCA1	I1405P	?	?
BRCA1	I1405V	?	✓
BRCA1	L1407P	?	✗
BRCA1	M1411T	?	✗
BRCA1	A1412P	?	✗
PALB2	L24F	?	✓
PALB2	K30N	?	✓
PALB2	K30P	?	✗
PALB2	K35P	?	✗
PALB2	S59E	?	✓✓
PALB2	S64E	?	✓✓

Above: We characterize whether mutations effect the structure of BRCA1 or PALB2 via CD and its effect on successful BRCA/PALB2 heterodimer formation via ITC.

Future Directions and Conclusions

- Remake BRCA1 T1394E mutation to test binding affinity to PALB2 through ITC.
- BRCA1 proline mutations disrupt alpha helix formation.
- There are no biologically significant binding differences between PALB2 WT, PALB2 S64E, or PALB2 S59E *in vitro*.
- PALB2 phosphorylation is not important to recreate when testing the effects of PALB2 variants of unknown significance (VUSs) on the BRCA1-PALB2 binding interaction *in vitro.*
- Further research is needed to determine the effect of BRCA1 phosphorylation on the BRCA1-PALB2 binding interaction *in vitro*.

References and Acknowledgements

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