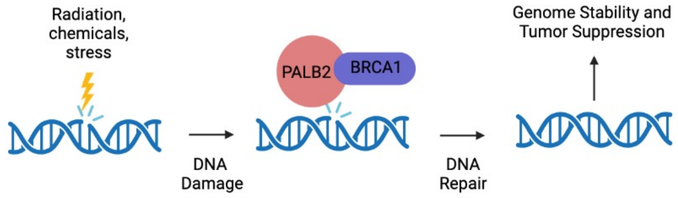
Investigating the Effects of BRCA1 Threonine Phosphorylation on PALB2 Interaction

Chloe Duvak and Mikaela D. Stewart Texas Christian University, Fort Worth, TX

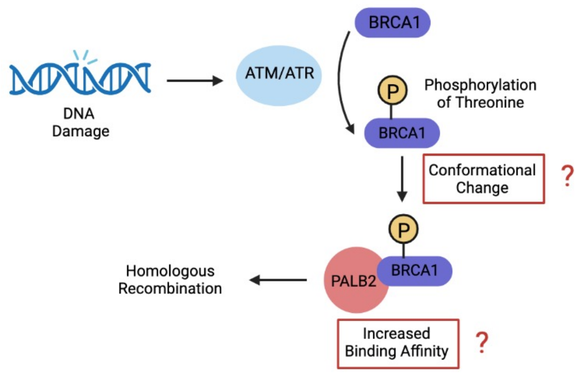
Introduction

BRCA1 and PALB2 function in homologous recombination.



Above: Upon DNA damage, BRCA1 and PALB2 form a heterodimer which functions to repair damaged DNA. Disruption of this interaction can result in development of mammary tumors [1].

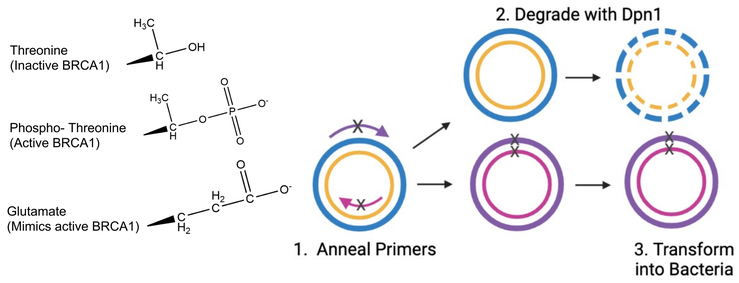
Phosphorylation could potentially act as an "on-switch" for protein interaction.



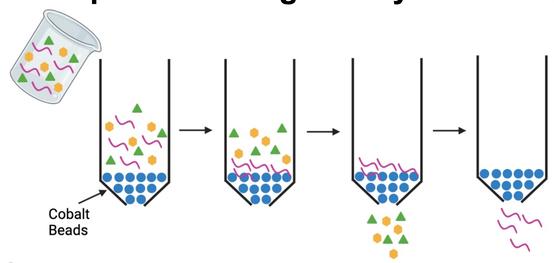
Above: Research has shown that phosphorylation of BRCA1 at specific sites, including T1394, promote the DNA damage response [2]. We predict a mechanism that phosphorylation promotes a conformational change in BRCA1 which leads to increased binding affinity with PALB2

Methods

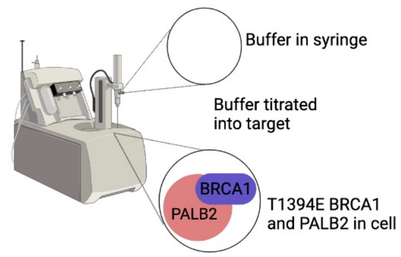
Mutagenesis was used to create a phosphomimicking mutant.



Protein is purified using affinity chromatography.

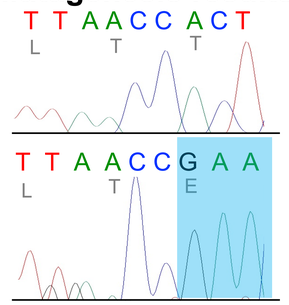


ITC is used to measure protein interaction.



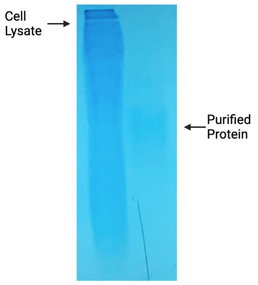
Results

Mutagenesis results

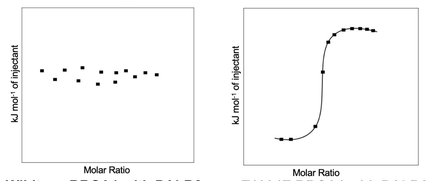


Protein purification results

Right: Cell lysate (unpurified protein) on left, purified protein on right.



Expected ITC results



Wildtype BRCA1 with PALB2 **T1394E BRCA1 with PALB2**

Above: We predict Wildtype BRCA1 will have little to no binding affinity to PALB2 (left). BRCA1 with the T1394E mutation will have a relatively high binding affinity in vitro (right).

Objectives

- Create a phosphomimic mutant at T1394 site in BRCA1 using mutagenesis.
- Purify mutated BRCA1 and wildtype PALB2 protein using affinity chromatography.
- Measure interactions between BRCA1 and PALB2 using ITC.

Future Directions

- Measure ITC data to prove our hypothesis.
- Measure binding affinity and interaction between T1394E BRCA1 and PALB2 with methods such as NMR (Nuclear Magnetic Resonance Spectroscopy) and CD (Circular Dichroism) to better understand this interaction.
- Investigate purpose of phosphorylation at other sites such as S1423E.

Acknowledgements

References:
Foo, T. K., Vincelli, G., Huselid, E., Her, J., Zheng, H., Simhadri, S., Wang, M., Huo, Y., Li, T., Yu, X., Li, H., Zhao, W., Bunting, S. F., & Xia, B. (2021). ATR/ATM-mediated phosphorylation of BRCA1 T1394 promotes homologous recombinational repair and G2–M Checkpoint Maintenance. *Cancer Research*, *81*(18), 4676–4684.

Pulver, E. M., Mukherjee, C., et al. (2021). A BRCA1 coiled-coil domain variant disrupting PALB2 interaction promotes the development of mammary tumors and confers a targetable defect in homologous recombination repair. *Cancer Research*, *81*(24), 6171–6182.

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