Evaluating the interaction between BRCA1 and estrogen receptor alpha

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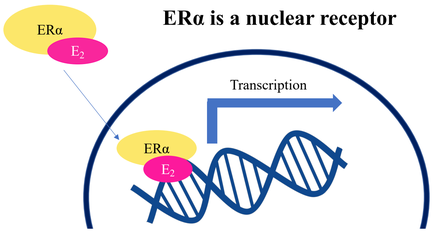
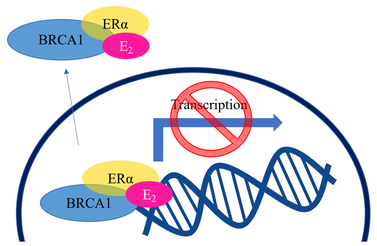
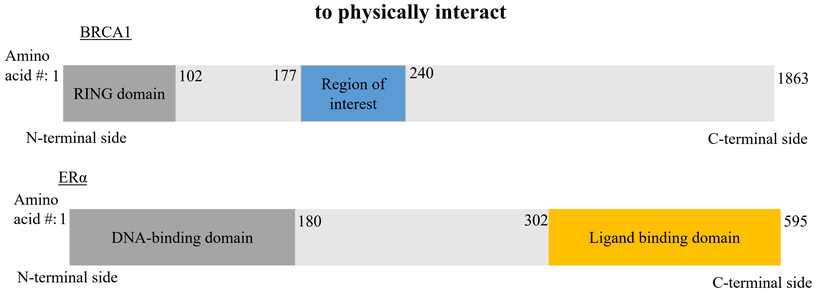
Introduction





Above: Estrogen receptor alpha (ERα) is a nuclear receptor, meaning that it is a protein whose job is to bind to estrogen responsive elements on DNA and induce transcription of estrogen-responsive genes. Breast cancer type 1 susceptibility protein (BRCA1) is a tumor suppressor protein found throughout all our cells and is essential in many cellular functions, including DNA repair, initiation of apoptosis, and regulation of gene transcription. In the case of the estrogen response pathway, BRCA1 regulates the transcription of estrogen-responsive genes turned on by ERα. If a mutation were to occur that interrupted the ability for BRCA1 to mediate genes transcription by ERα, a higher risk of breast, ovarian, and prostate cancer results [1, 2].

ERα ligand binding domain (LBD) and amino acids 177-240 of BRCA1 are theorized to physically interact

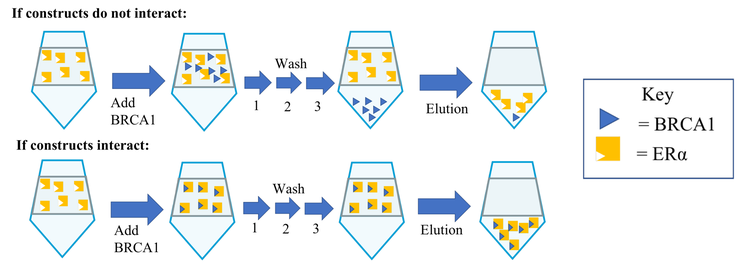


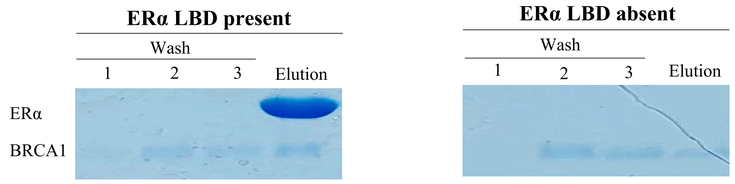
Above: Although the RING domain found on the N-terminus of BRCA1 is where the ubiquitylation function is found, and ubiquitylation of the DNA-binding domain suppresses its ability to induce transcription, Previous studies have isolated amino acids 177-240 of BRCA1 and the ERα LBD, which are thought to be involved in binding and are indicated above by the colors blue and yellow, respectively [3]. The protein constructs used in this research consist only of the amino acids making up these two regions of interest.

Objectives

- Using in vitro methods, document the molecular details of the estrogen receptor region of BRCA1 binding to estrogen receptor alpha, both in the presence and absence of estrogen.
- Determine biochemical and biophysical methods conducive to studying this interaction.

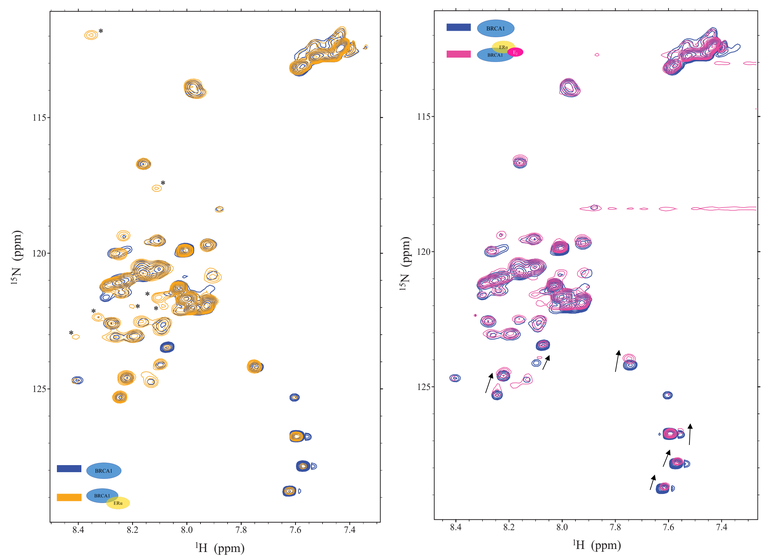
ERα and BRCA1 interact *in vitro*





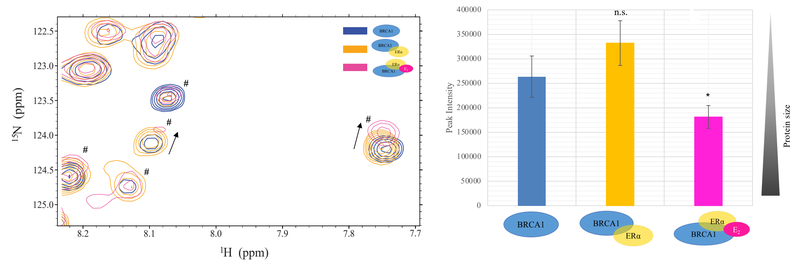
Above: Gel electrophoresis results of a pull-down assay confirming qualitative binding between the shortened BRCA1 and ERα constructs studied.
Left: The greatest volume of BRCA1 was found after elution with ERα LBD present in the resin.
Right: Control pull down; The greatest volume of BRCA1 was found in wash 2 without ERα in the resin.

Estrogen impacts ERα-BRCA1 interaction



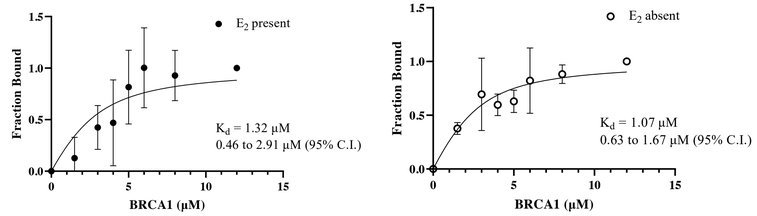
Nuclear magnetic resonance (NMR) spectra of BRCA1 and BRCA1 + ERα (left): The appearance of new peaks in BRCA1 spectra with the addition of ERα to the sample (denoted with an asterisk, *) indicates an interaction between the two protein constructs.
NMR spectra of BRCA1 and BRCA1 + ERα + E_2 (right): Additional visible chemical shifts in BRCA1 amino acids in the presence of both estrogen (E_2) and ERα (denoted with an arrow, →). The shift in chemical environment of certain peaks in the presence of ERα and E_2 implies an improved interaction compared to the addition of ERα only.

Addition of estrogen decreases NMR peak intensity



Comparison of NMR of BRCA1 (blue) to the sample including ERα (yellow) and the sample including ERα and E_2 (pink).
Left: Addition of ERα only slightly alters peak distribution and intensity, while the presence of E_2 leads to more significant chemical shifts and a decrease in peak intensity, indicated by an arrow (→) and pound sign (#), respectively.
Right: Quantification of the average peak intensity of each sample using a two-tailed *t* test ($P < 0.05$) compared against the APO sample. An asterisk (*) signifies a change in average peak intensity with a P-value < 0.05, and n.s. denotes no significance.

No significant difference in K_d found with and without E_2



Binding curve and dissociation constant (K_d) of the ERα-BRCA1 system (above): Each graph plots the fraction of ERα bound to BRCA1 against the concentration of BRCA1 found. A binding curve was derived using data from a fluorescence quenching experiment. A nonlinear regression curve derived from a binding equilibrium equation was used to find K_d [4]. No significant difference was found between the K_d of the ERα–BRCA1 system in the presence and absence of E_2 in these experimental conditions.

Conclusions

- A pull-down assay and NMR spectroscopy confirmed binding between the ERα LBD and BRCA1 constructs used.
- Quantification of NMR average peak intensity indicates that the presence of estrogen leads to a stronger binding affinity between ERα and BRCA1, but fluorescence quenching experiments did not find any significant difference in the binding coefficients.
- Additional fluorescence quenching is required to better understand binding affinity

References and Funding

1. Wang L, Di LJ. BRCA1 and estrogen/estrogen receptor in breast cancer: where they interact? Int J Biol Sci. 2014 May 14;10(5):566-75.
2. 2. Ma Y, Katiyar P, Jones LP, Fan S, Zhang Y, Furth PA, Rosen EM. The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. Mol Endocrinol. 2006 Jan;20(1):14-34.
3. Eakin CM, Maccoss MJ, Finney GL, Klevit RE. Estrogen receptor alpha is a putative substrate for the BRCA1 ubiquitin ligase. Proc Natl Acad Sci U S A. 2007 Apr 3;104(14):5794-9.
4. Stewart M.D., Morgan B., Massi F., Igumenova T.I. Probing the determinants of diacylglycerol binding affinity in the C1B domain of protein kinase Cα. J Mol Biol. 2011 May 20;408(5):949-70.

Thank you to the TCU College of Science and Engineering for funding of this project.

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