Investigating the molecular details of the interaction between 2 tumor suppressing proteins

Khoa Dao, Mikaela Stewart
Department of Biology, Texas Christian University, Fort Worth, TX

Background

- Breast cancer type 1 susceptibility protein (BRCA1) is involved into many essential cellular processes, including tumor suppression, DNA damage sensors and signaling, DNA repair of double-strand DNA breaks. Inherited mutations in BRCA1 can result in up to 80% increased risk of breast and ovarian cancer. [1]
- Tumor protein P53 is crucial for multicellular organisms, in which P53 prevent cancer formation by regulating gene expression and preserving genome stability. The encoding TP53 gene is mutated in up to 50% of human cancer cases. [2]
- BRCA1 and P53 have been found to bind with one another in vivo. However, the exact binding region and the amino acid residues involved in the interaction remain unclear.

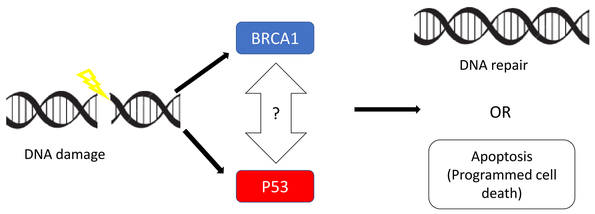


Figure 1. BRCA1 and P53 has essential anti-tumor functions

Objectives

1) Investigate whether the is a physical interaction between BRCA1 and P53 in vitro

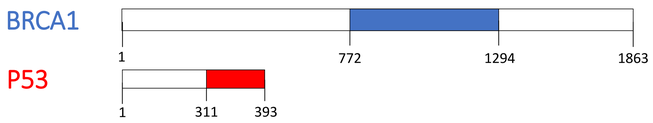


Figure 2.1 The blue and red regions were shown to interaction in cells by an immunoprecipitation study [3], but there is no evidence that they directly interact.

2) Narrow down the binding region between the two proteins

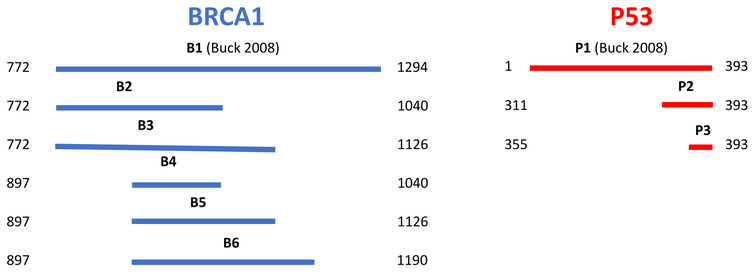
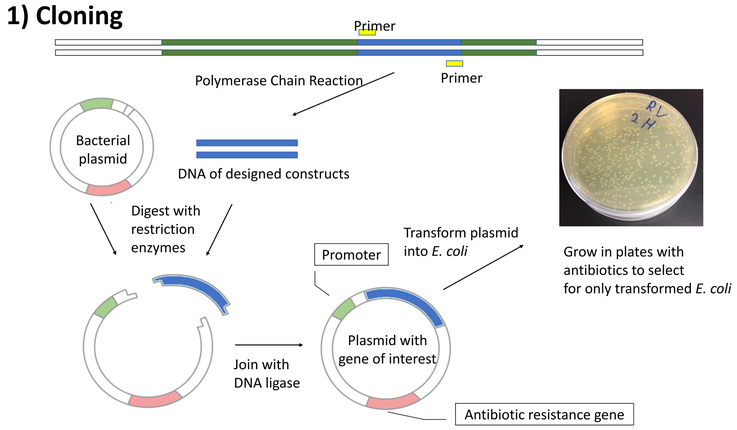


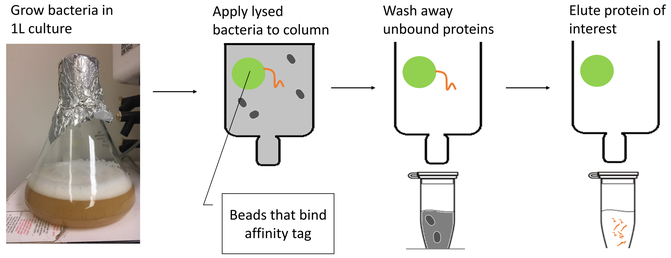
Figure 2.2 BRCA1 and P53 protein constructs are designed based on the amino acid Proline, which indicates the possible presence of structure.

Protein constructs expression

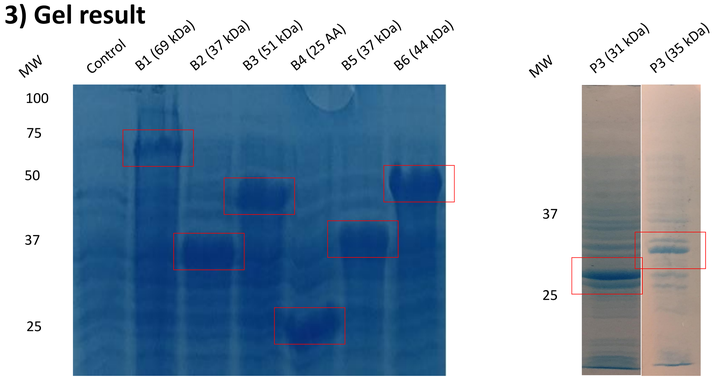
1) Cloning



2) Expression and purification

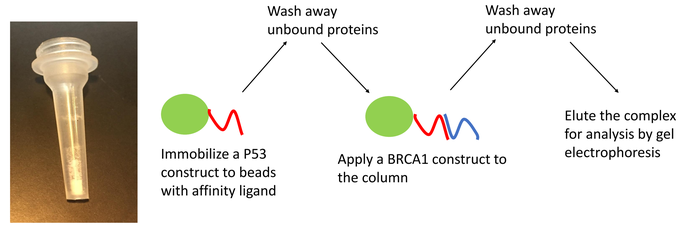


3) Gel result



Gel result for BRCA1 and P53 constructs. (MW = molecular weight)

Pull down assay



Result

Summary

- Cloned, expressed and purified BRCA1 and P53 protein constructs

Future direction

- Optimize the conditions for expression and purification of BRCA1 and P53 protein constructs and perform pull down assay.
- Utilize NMR spectroscopy to identify specific amino acids involved in the interaction once the binding regions have been narrowed down

Acknowledgement

This project was funded by SERC
Special thanks to my committee members Dr. Stewart, Dr. Jeffries and Dr. Dzuyba, as well as the members in the Stewart lab

Reference:

[1] Bourdon, J., Surget, S., & Khoury, M. (2013). Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. *Oncotargets And Therapy*, 57. doi: 10.2147/ott.s53876

[2] Buck, M. (2008). A novel domain of BRCA1 interacts with p53 in breast cancer cells. *Cancer Letters*, *268*(1), 137-145. doi: 10.1016/j.canlet.2008.03.061