

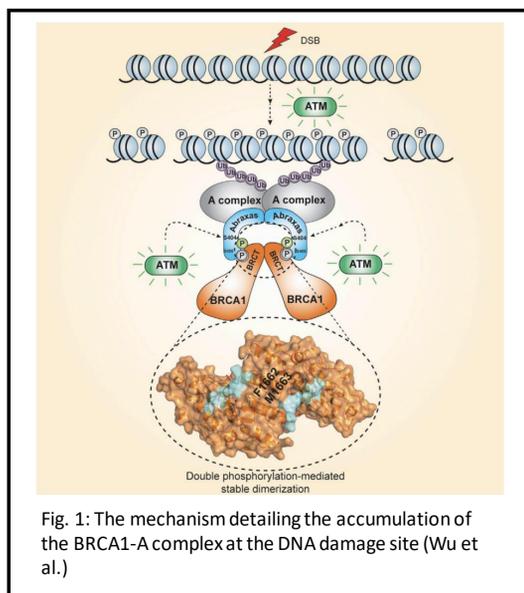
Characterizing the Abraxas/BRCA1 Interaction in the Control of DNA Double Strand Breaks

Grace Murphy, Xiao Wu, Shengfeng Xu, Bin Wang

Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction

One of the hallmarks of cancer is genomic instability. Upon DNA damage, multiple repair pathways exist, one of which being homologous recombination (HR). This pathway uses the sister chromatid as a template to repair the DNA double strand break (DSB) and is mediated by BRCA1. The BRCA1 gene is a crucial player in stabilizing genome integrity. Germline breast and ovarian cancer show high rates of mutation of this gene. HR is initiated by DNA end resection, creating a long 3' ssDNA tail that can invade the homologous DNA strand. The BRCA1-A complex (Figure 1) is a collection of proteins that work together to repair DSB. Two crucial proteins of this complex are Abraxas, a scaffold protein, and BRCA1. When DNA is damaged, ATM signaling induces ubiquitination of lysines conjugated at the damage site. RAP80, a protein in the BRCA1-A complex, binds to the polyubiquitin chain of lysine 63, recruiting BRCA1. Abraxas then dimerizes and stabilizes BRCA1 at the site, initiating HR.



It has been shown that DNA end resection as a result of camptothecin (CPT) treatment, a topoisomerase inhibitor, is inhibited by Abraxas, limiting break-induced replication (BIR). BIR is a highly mutagenic repair pathway that is initiated by the collapse of the replication fork and telomere erosion. Crucial to its function as a tumor suppressor is BRCA1's ability to recognize pSPxF motifs on other proteins through its two BRCT domains. Abraxas and BRCA1 interact at two important points when they form the BRCA1-A complex: S406 and S404. S406 is in the SPxF motif in Abraxas's C-terminus, is phosphorylated in response to DNA damage, and interacts directly with the BRCT domain of BRCA1. S404 is near the SPxF motif in Abraxas's C-terminus. Its phosphorylation is required for dimerization of BRCA1-BRCT/Abraxas and is crucial for BRCA1 accumulation at DNA damage sites. Double phosphorylation of S404S406 is induced by ionizing radiation in an ATM pathway dependent manner.

Materials

- Cell lines: U2OS (untreated and treated), ULF3 (Abraxas KO), ULF3 WT, ULF3 S404S406 double mutant
- 1 μ M CPT
- FiberPrep® DNA Extraction Kit
- FiberComb® Molecular Combing System
- BrdU antibody
- Fluorescence dye 488
- 80i microscope
- ImageJ software

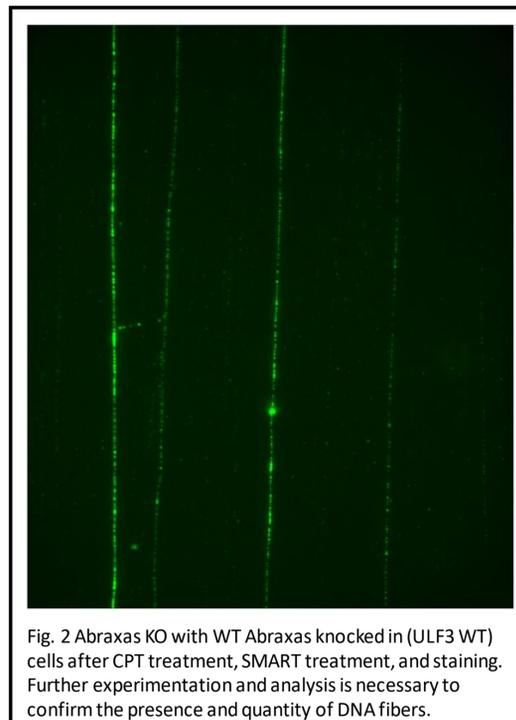


Fig. 2 Abraxas KO with WT Abraxas knocked in (ULF3 WT) cells after CPT treatment, SMART treatment, and staining. Further experimentation and analysis is necessary to confirm the presence and quantity of DNA fibers.

Methods

- Culture U2OS cells to have four lines: U2OS, Abraxas KO (ULF3), ULF3 with WT Abraxas knocked in, ULF3 with S404S406 double mutant knocked in
- Upon 40-50% confluency, treat cells for 24-48 hours with BrdU
- Treat cells for 1 hour with 1 μ M CPT
 - The U2OS cell lines should have two treatments: CPT treatment and no CPT treatment
- Count cells and calculate appropriate volume to all for 1 x 10⁶ cells/plug
- Form plugs
 - Plugs are fragile; it is recommended to have multiples of each treatment
- Digest proteins using FiberPrep® DNA Extraction Kit Digestion Buffer and wash
- Prepare DNA solution for FiberComb®

- Comb DNA onto coverslips and bake
- Adhere coverslips to slides
- Using the 80i microscope, take images of the DNA fibers
- Use ImageJ software to measure the lengths of the fibers
- Compile the data and statistically analyze using ANOVA
- Plot the mean fiber length for each treatment

Conclusions

Based on these preliminary findings, the interaction of BRCA1 and Abraxas at these interaction points cannot be conclusively determined. The images produced did not show distinct ssDNA fibers, making quantification impossible. Further experimentation is needed to confirm the results.

Future Directions

- Test if Abraxas S404S406 double mutant affects Abraxas's function in inhibition of DNA end resection and BIR in response to CPT damage
- Survey BIR levels using MiDAS
- Analyze known Abraxas cancer mutations using SMART and MiDAS

References

- 1) Ferrara, Luciana, and Eric B Kmiec. "Camptothecin Enhances the Frequency of Oligonucleotide-Directed Gene Repair in Mammalian Cells by Inducing DNA Damage and Activating Homologous Recombination." *Nucleic Acids Research*, vol. 32, no. 17, 2004, pp. 5239-5248., doi:10.1093/nar/gkh822.
- 2) Futreal, P.A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L.M., haugen-Strano, A., Swensen, J., Miki, Y., et al. (1994). BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266, 120-122.
- 3) Marco Muzi-Falconi and Grant W. Brown (eds.), *Genome Instability: Methods and Protocols*, Methods in Molecular Biology, vol. 1672, DOI 10.1007/978-1-4939-7306-4_12, ©Springer Science+Business Media LLC 2018
- 4) Wu et al., 2016, *Molecular Cell* 61, 434-448 February 4, 2016 ©2016 The Authors
<http://dx.doi.org/10.1016/j.molcel.2015.12.017>