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FANCA KO Breast Cancer Cells are Sensitized to PARPi and ATRi by Homologous Recombination-Independent Mechanisms

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Introduction – An *in vivo* CRISPR Screen Identified Loss of FANCA as Synthetic Lethal with PARPi

- Synthetic Lethality – when two genetic alterations are viable alone but lethal in combination
- *In-vivo* CRISPR screens recapitulate the complex tumor microenvironment and select for synthetic lethal mutations compatible with tumorigenesis
- FANCA is mutated or deleted in 4.2% of human metastatic breast cancers[1]

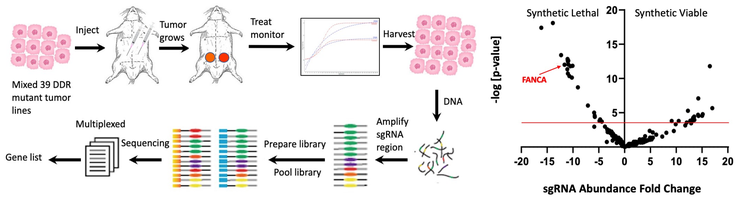

Figure 1. Left: Workflow for an *in vivo* CRISPR screen to detect mutations that confer sensitivity to PARPi or ATRi (Q. Wang). Right: Volcano plot of gene that are synthetic lethal or synthetic viable with PARPi when knocked out via CRISPR/Cas9.

Loss of FANCA – But Not Loss of Interacting or Downstream FA Proteins – Confers PARPi Sensitivity in Cancer Cells

- FANCM is one of several proteins (including FANCA) that make up the FA core complex
- FANCD2 binds damaged dsDNA and recruits DNA repair proteins after monoubiquitination mediated by the FA core complex

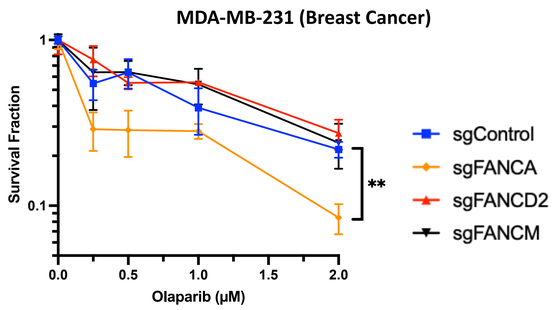

Figure 2. Results of a clonogenic viability assay show that FANCA-deficient breast cancer cells, but not FANCM- or FANCD2-deficient breast cancer cells, are sensitized to the PARPi Olaparib.

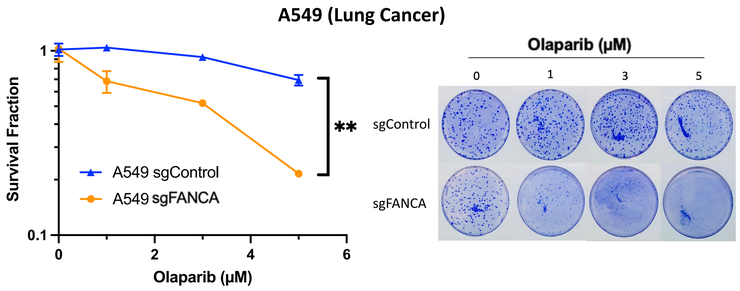

Figure 3. Results of a clonogenic viability assay show that FANCA-deficient lung cancer cells (A549) are sensitized to the PARPi Olaparib.

PARPi and ATRi Act Synergistically in FANCA KO Breast Cancer and Lung Cancer Cells

- Synergy Score (**δ**) = % Response beyond expected if drugs were additive
- Interpreting Synergy Scores
  - Larger than 10: the interaction between two drugs is likely to be **synergistic**
  - From -10 to 10: the interaction between two drugs is likely to be **additive**
  - Less than -10: the interaction between two drugs is likely to be **antagonistic**

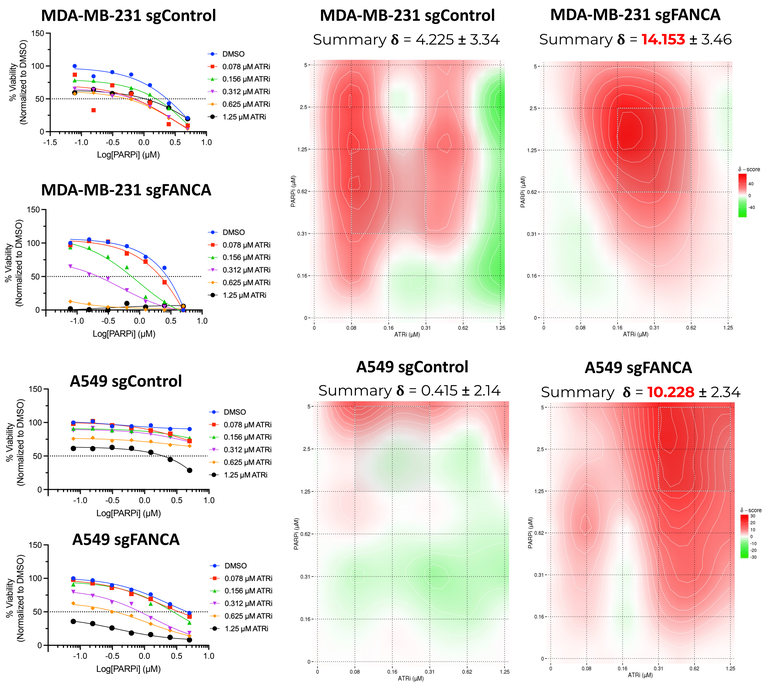

Figure 4. CellTiter-Glo® viability assays show that PARPi and ATRi act synergistically to reduce cell viability in FANCA KO breast and lung cancer cells. Heatmaps generated using SynergyFinder software.[2]

FANCA KO Breast Cancer Cells are Proficient in Homologous Recombination

- γH2AX is a marker of DNA damage
- Rad51 is a marker of homologous recombination repair

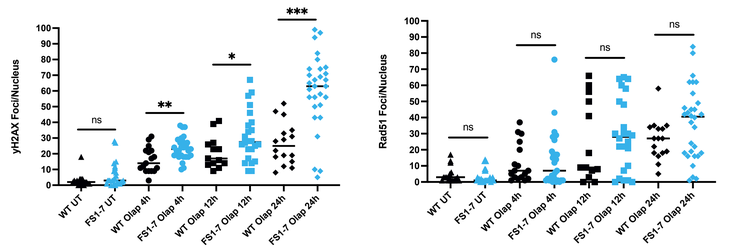

Figure 5. IF detection of nuclear foci of Rad51 and γH2AX in FANCA KO vs. sgControl (FANCA WT) cells. (p >0.05 = ns; p <0.05 = *, p <0.01 = **; p <0.001 = ***; p <0.0001 = ****)

FANCA KO Breast Cancer Cells Display Increased PARP1 Activity Under Replication Stress

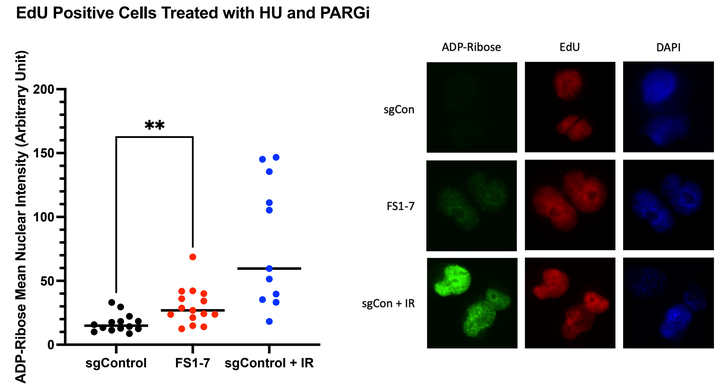

Figure 6. IF detection of nuclear intensity of ADP-Ribose in FANCA KO vs. sgControl cells. (p >0.05 = ns; p <0.05 = *, p <0.01 = **; p <0.001 = ***; p <0.0001 = ****)

Future Directions

- Perform PARPi + ATRi combination assay on RPE-1 cells +/- inducible expression of Myc or Cyclin E to investigate the hypothesis that oncogenic signaling is important for PARPi and ATRi sensitivity in FANCA KO cells
- Perform clonogenic viability assays in the presence of PARPi or ATRi on cancer cells expressing various mutant alleles of FANCA to determine the domains of FANCA which are important for protection against PARPi and ATRi toxicity

References

1. Rose, M., Burgess, J. T., O'Byrne, K., Richard, D. J., & Bolderson, E. (2020). PARP inhibitors: Clinical relevance, mechanisms of action and tumor resistance. *Frontiers in Cell and D*The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discovery*. 2017;7(8):818-831. Dataset Version 8.

2. Ianevski, A., Giri, K. A., Aittokallio, T., 2022. SynergyFinder 3.0: an interactive analysis and consensus interpretation of multi-drug synergies across multiple samples. Nucleic Acids Research.

Acknowledgements

We are grateful to Dr. Pablo Ariel and the Microscopy Services Laboratory staff for assistance with immunofluorescence imaging. We also thank Jon DeLiberty for assistance with CellTiter-Glo® assays, as well as the members of the Gupta Lab for helpful feedback. This project is supported by BCRF Grant 5119001.