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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 - What Gene Mutations Can be Targeted with PARP Inhibitors in Treatment-Refractory Breast Cancers?

- An *in vivo* CRISPR screen was performed to **detect DNA Damage Response gene mutations that sensitize breast cancer cells to PARP inhibitor (PARPi) or ATR inhibitor (ATRi)**, anti-cancer drugs that kill cells by inducing DNA damage.[1,2]
- Unlike *in vitro* screens, this *in vivo* screen selected for sensitizing mutants which are **compatible with tumor viability** and thus of greater clinical relevance.

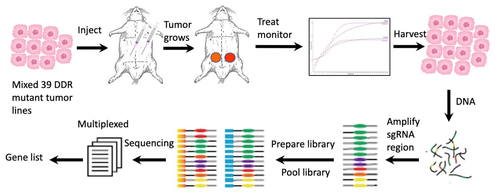

Figure 1: Workflow for an *in vivo* CRISPR screen to detect mutations that confer sensitivity to PARPi or ATRi (Q. Wang).

- Small guide RNAs (sgRNAs) were engineered to allow CRISPR-Cas9 to target and **cleave genes of interest** in cells treated with PARPi or ATRi. **Low sgRNA abundance** compared to wild type (WT) indicates that **the gene mutation caused a reduction in cell viability,** and therefore **sensitizes cells to the treatment.**
- Loss of **FANCA**, a DNA Damage Response gene **mutated in ~3% of human cancers,**[3] was identified in the *in vivo* screen to confer **sensitivity to both PARPi and ATRi.**

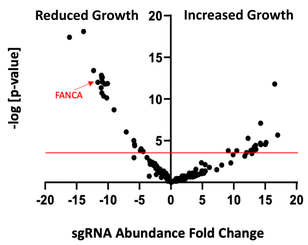

Figure 2: Volcano plot of genes that, when mutated, conferred sensitivity to PARPi. FANCA was identified as a clinically relevant mutation which confers a high degree of PARPi-sensitivity with high statistical significance, indicated by low p-value (Q. Wang).

FANCA KO Cells are Sensitive to PARPi and ATRi

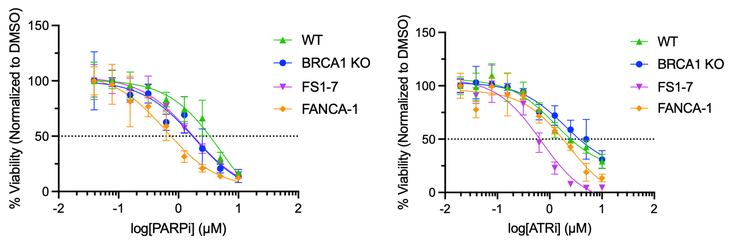

Figure 3: Dose response curves of PARPi and ATRi treatment in WT, BRCA1 KO, and two FANCA KO (FS1-7, FANCA-1) cell lines. Curves generated by CellTiter-Glo® assays.

Cell Line	PARPi GI_{50} (μM)	ATRi GI_{50} (μM)
WT	3.3	2.5
BRCA1 KO	1.7	3.6
FS1-7	1.8	0.6
FANCA-1	0.7	1.8

Figure 4: GI_{50} values (dose at which cell growth is inhibited by 50% compared to untreated control) for PARPi and ATRi treatment. Lower GI_{50} corresponds to higher drug sensitivity.

PARPi and ATRi act Synergistically in FANCA KO cells

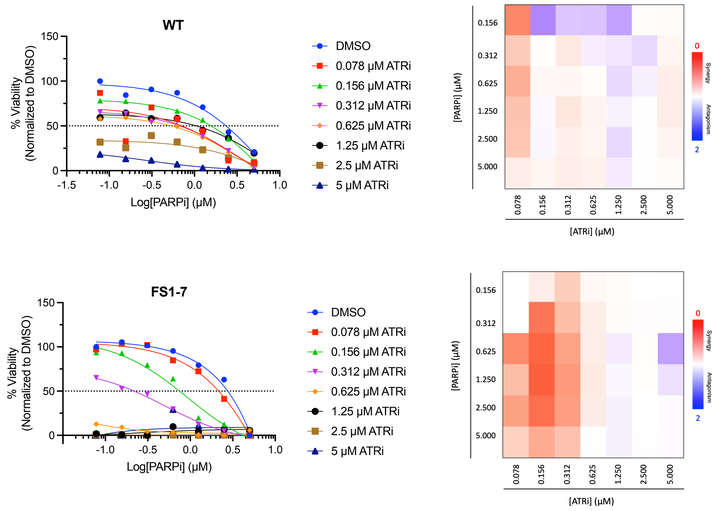

Figure 5: CellTiter-Glo® viability assays show that PARPi and ATRi act synergistically to reduce cell viability in FANCA KO cells. Heatmaps generated in R.

FANCA KO Cells are Proficient in Homologous Recombination

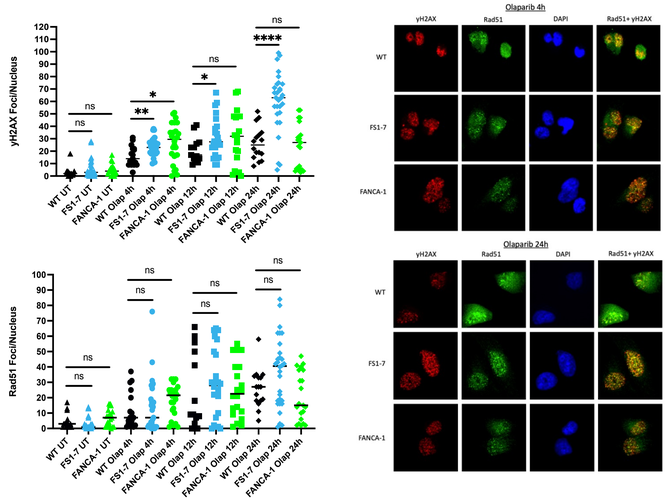

Figure 6: IF detection of nuclear foci of Rad51 and γ-H2AX in FANCA KO vs. WT cells. (p >0.05 = ns; p <0.05 = *, p <0.01 = **; p <0.001 = ***; p <0.0001 = ****)

Synergy Calculation Methodology

$$\text{Bliss Synergy Score} = \frac{\text{Expected Kill Effect if Additive}}{\text{Observed Kill Effect}}$$

Kill Effect (KE) = reduction in viability with respect to UT

Expected Kill Effect if Additive = $KE_1 + KE_2 - (KE_1)(KE_2)$

Conclusions

- FANCA mutations are **highly prevalent in human cancers** and show **high synergistic sensitivity to PARPi and ATRi**, making them **promising candidates for clinical investigation of PARPi and ATRi therapy.**
- While PARPi induces increased DNA damage in FANCA KO cells, this **increased damage is not caused by HR deficiency** since FANCA KO cells are proficient at forming Rad51 foci following DNA-damaging treatment. **These findings challenge the traditional hypothesis that PARPi sensitivity is conferred by deficiency in HR.**

Future Directions

- Perform PARPi + ATRi synergy assays on other cancer cell lines, including lung cancer (A549, H1299), colorectal cancer (HCT116), and an additional breast cancer line (MCF7). This will allow for further evaluation of the potential clinical impact of PARPi + ATRi combination therapy.
- Perform IF staining of ADP-Ribose, the product of PARP1, in WT and FANCA KO breast cancer cells to evaluate the hypothesis that under replication stress, PARP1 has higher activity in FANCA KO cells than in WT cells due to loss of FANCA's supporting role in Okazaki fragment maturation.

References

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