Investigating the ACSL4 dependency in recurrent tumor cells

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Background

Breast cancer recurrence is a driver in cancer patient death.

- Residual tumor cells survive in the breast for months to years prior to the establishment of recurrent tumors.
- 25-39% of HER2+ breast cancer results in recurrent disease[1].
- Recurrent tumor cells exhibit a distinct metabolism compared to primary tumor cells[2].

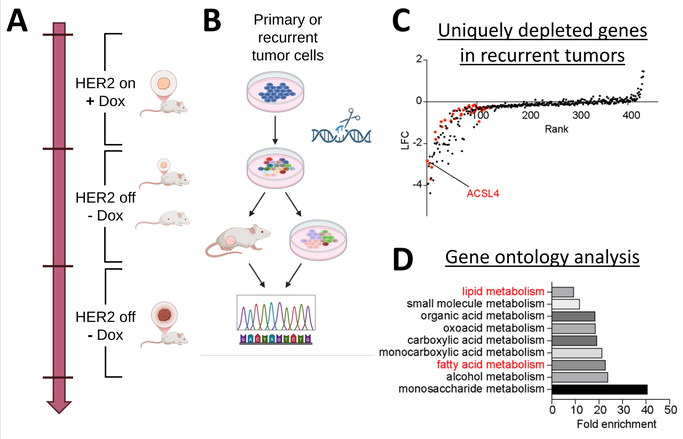


Figure 1. (A) Transgenic Her2-driven mouse model was used to establish both primary (+ doxycycline; dox) and recurrent (- dox) tumors. These tumors were digested for cell line formation. **(B)** Schematic of CRISPR metabolism screen performed in both primary and recurrent cells. At tumor endpoint (*in vivo*) or after 14 population doublings (*in vitro*) DNA was isolated for next generation sequencing. **(C)** Ranked long fold change (LFC) plot of genes uniquely depleted in recurrent tumors. **(D)** Gene ontology analysis of genes uniquely upregulated in recurrent cells.

ACSL4 expression is essential for recurrent tumor cell survival but not primary tumor cells both *in vitro* and *in vivo*.

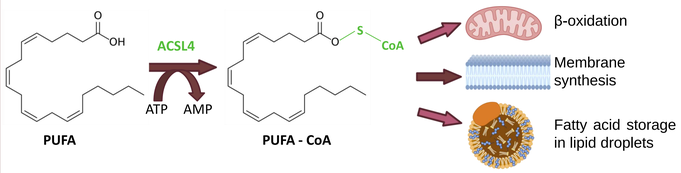


Figure 2. ACSL4 attaches a coenzyme A (CoA) group to a polyunsaturated fatty acid (PUFA) for downstream signaling including: β-oxidation, membrane synthesis, and lipid droplet synthesis.

Methods

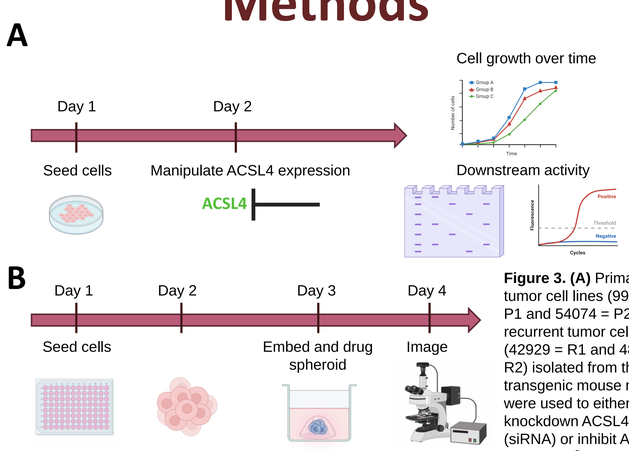
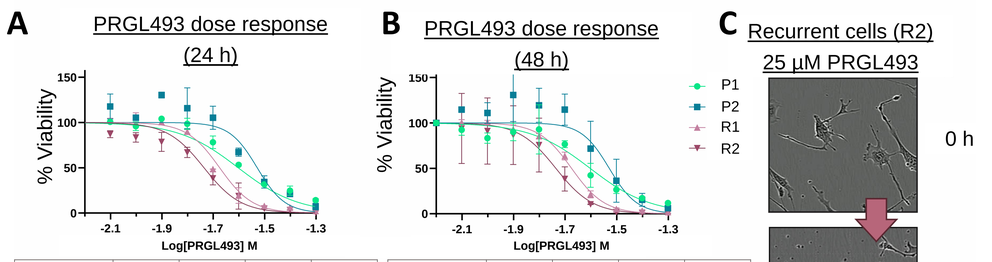


Figure 3. (A) Primary tumor cell lines (99142 = P1 and 54074 = P2) and recurrent tumor cell lines (42929 = R1 and 48316 = R2) isolated from the transgenic mouse model were used to either knockdown ACSL4 (siRNA) or inhibit ACSL4 (PRGL493[3]). Literature established IC50 using purified recombinant ACSL4 as 50 μM[3]. **(B)** 3D spheroid invasion assay workflow schematic.

Recurrent cells are uniquely sensitive to ACSL4 inhibition



Cell Line	P1	P2	R1	R2
IC50 (μM)	0.027	0.029	0.020	0.018
Average IC50	0.027		0.020	

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IC50 (μM)	0.027	0.029	0.020	0.018
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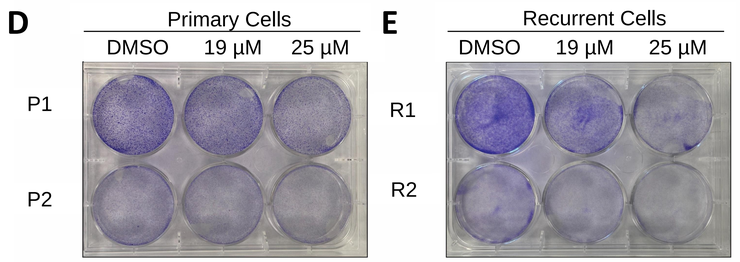

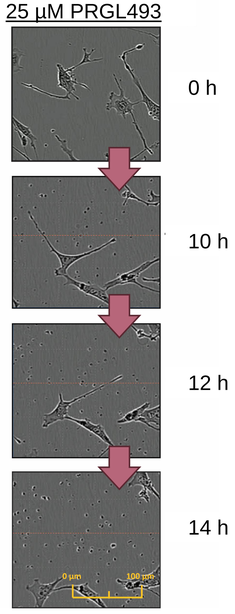

Figure 4. (A, B) ACSL4 dose response using Cell Titer Glo after (A) 24 hours or (B) 48 hours. **(C)** Recurrent cell line (48316, R2) time course upon 25 μM PRGL493 treatment. Images taken using Incucyte. **(D, E)** Crystal violet staining after 24 hours using recurrent cell line IC50 (19 μM) or IC70 (25 μM).

Reduced 3D cellular invasion upon ACSL4 inhibition

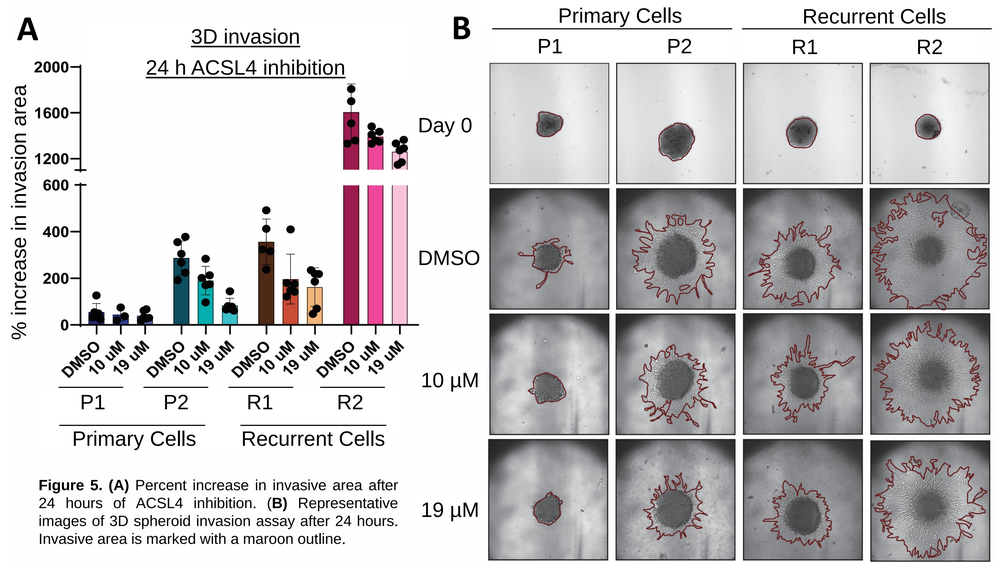

Figure 5. (A) Percent increase in invasive area after 24 hours of ACSL4 inhibition. **(B)** Representative images of 3D spheroid invasion assay after 24 hours. Invasive area is marked with a maroon outline.

ACSL4 inhibition hints at dysregulated autophagic flux

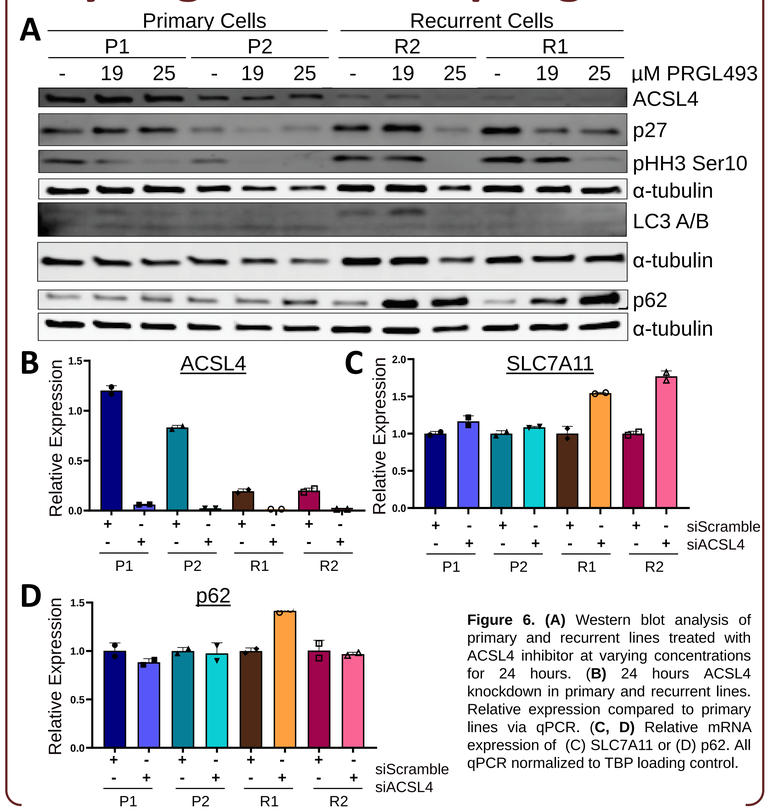

Figure 6. (A) Western blot analysis of primary and recurrent lines treated with ACSL4 inhibitor at varying concentrations for 24 hours. **(B)** 24 hours ACSL4 knockdown in primary and recurrent lines. Relative expression compared to primary lines via qPCR. **(C, D)** Relative mRNA expression of (C) SLC7A11 or (D) p62. All qPCR normalized to TBP loading control.

Conclusions

- Recurrent cells exhibit a lower IC50 compared to primary cells.
- Recurrent AND primary cells exhibit a dose dependent decrease in 3D invasion upon ACSL4 inhibition.
- Recurrent cells dose dependently increase p62 protein and SLC7A11 mRNA upon ACSL4 inhibition.

Future Directions

- Determine whether ACSL4 inhibition is halting autophagy, upregulating NRF2 signaling, or both in recurrent cells.
- Assess whether ACSL4 localizes to autophagosomes specifically in recurrent cells to drive autophagy and maintain cell survival.

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