EGLN1 is a synthetic lethal target in *ARID1A*-mutant ovarian cancer

ABSTRACT# 2892

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ABSTRACT

The successful use of PARP inhibitors for the treatment of BRCA-mutant ovarian cancer underscores the importance of synthetic lethality as a therapeutic opportunity for tumors with loss-of-function mutations in tumor suppressor genes. Ovarian cancer ranks as the fifth deadliest cancer among women, and one ovarian cancer subtype, clear cell carcinoma, is particularly underserved. The most frequent genetic alteration in ovarian clear-cell carcinoma is loss-of-function mutation of the SWI/SNF-A complex subunit, *ARID1A*. We interrogated the data published by Project Achilles to determine candidate synthetic lethal partners with *ARID1A* in ovarian cancer, and identified *EGLN1*, a member of the EGLN-family of prolyl hydroxylases. Inhibition of EGLN1, either genetically or pharmacologically, leads to decreased viability in ovarian cancer cell lines. Pharmacological inhibition of EGLN1 is clinically achievable as evidenced by several well-tolerated, small molecule inhibitors currently in clinical trials for the treatment of anemia in the context of chronic kidney disease. The synthetic lethal interaction of *ARID1A* and *EGLN1* may provide a therapeutic opportunity for patients diagnosed with ovarian clear-cell carcinoma.

EGLN proteins regulate HIFα stability

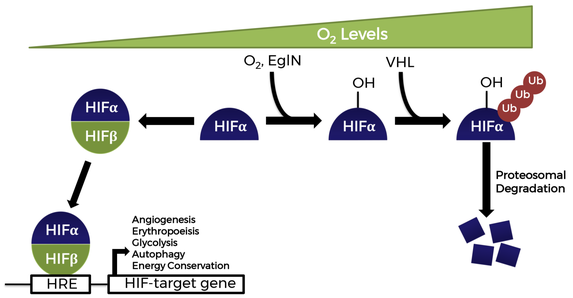

Figure 1: The EGLN family of prolyl hydroxylases regulates HIFα subunit stability. The EGLN family of prolyl hydroxylases (EGLN1-3) regulates HIFα stability by hydroxylating two conserved proline residues in the oxygen-dependent degradation domain of the HIFα subunits. Prolyl-hydroxylated HIFα is recognized by a ubiquitin ligase complex that includes VHL, and is targeted for rapid proteasomal degradation.

ARID1A is commonly mutated in OCCC

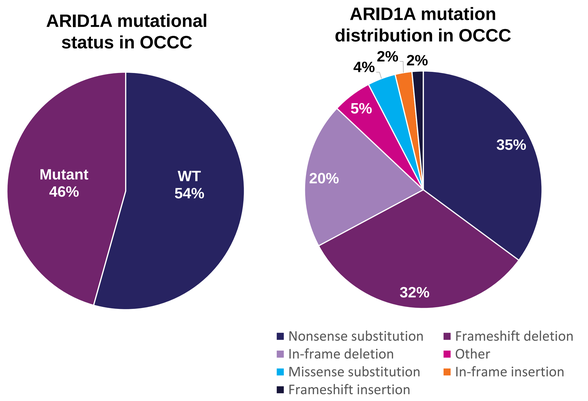

Figure 2: ARID1A mutation is one of the most frequent genetic alterations in ovarian clear cell carcinoma. Tumor sample-specific data was analyzed from the COSMIC database (cancer.sanger.ac.uk; Forbes et al. 2017) for the ARID1A mutational status in ovarian clear cell carcinoma (left panel), as well as the type of mutation (right panel). Note: the majority of ARID1A mutations are predicted to confer loss-of-function.

ARID1A^{mutant} ovarian cancer cell lines are EGLN1-dependent

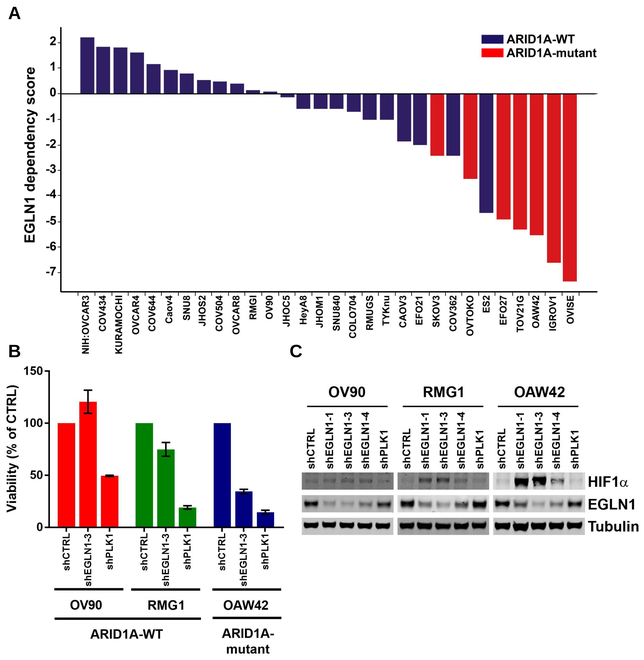

Figure 3: ARID1A-mutant ovarian cancer cell lines are dependent on EGLN1. (A) Data from the Project ACHILLES RNAi screens were interrogated for ovarian cancer cell line dependence on EGLN1. In brief, 30 ovarian cancer cell lines were included as part of the 501 cancer cell lines used in genome-wide, pooled loss of function screens targeted with ~100,000 shRNAs. DEMETER gene solutions were compiled to produce gene dependency scores (Cowley et al., 2014 and Tsherniak et al., 2017). (B) ARID1A-WT ovarian cancer cell lines (OV90 and RMG1) and an ARID1A-mutant ovarian cancer cell line (OAW42) were engineered to stably express doxycycline-inducible shRNAs (vector purchased from Cellecta, Inc) targeting EGLN1 or PLK1 (shEGLN1-3 and shPLK1, respectively), or a non-targeting control (shCTRL). Cells were induced with 1 μg/mL doxycycline for 2 weeks and stained with crystal violet. Data represents the quantification of 2 independent experiments. (C) Immunoblot of cell lines generated in (B), as well as two additional EGLN1 shRNAs (shEGLN1-1 and shEGLN1-4). Cells were induced with 1 μg/mL doxycycline for 72 hours.

EGLN1-dependent cell lines accumulate HIF1α

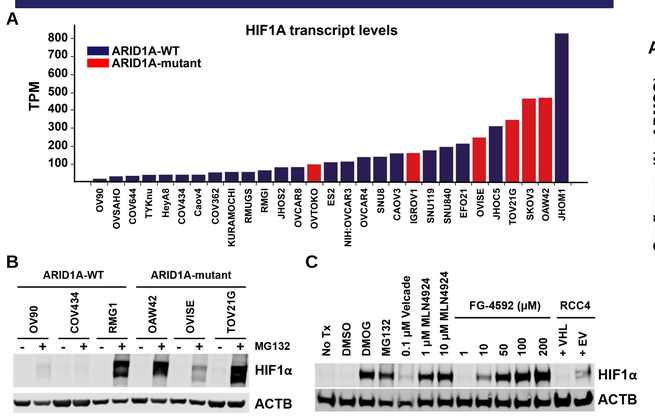

Figure 4: EGLN1-dependent cell lines accumulate high levels of HIF1α protein when the EGLN/VHL regulatory axis is inhibited. (A) RNAseq data quantifying HIF1A transcript levels in ovarian cancer cell lines. (B) Immunoblot of ARID1A-WT and ARID1A-mutant ovarian cancer cell lines treated with 10 μM MG132, a proteasomal inhibitor, for 4 hours. (C) Immunoblot of an ARID1A-mutant cell line (OVISE) treated for 4 hours with pan-EGLN inhibitors (DMOG and FG-4592), proteasomal inhibitors (Velcade and MG132), neddylation inhibitor (MLN-4924) or vehicle (DMSO). A VHL-null RCC isogenic cell line pair (RCC4) is included for comparison.

ARID1A-mutant ovarian cancer cells are sensitive to pharmacological EGLN inhibition

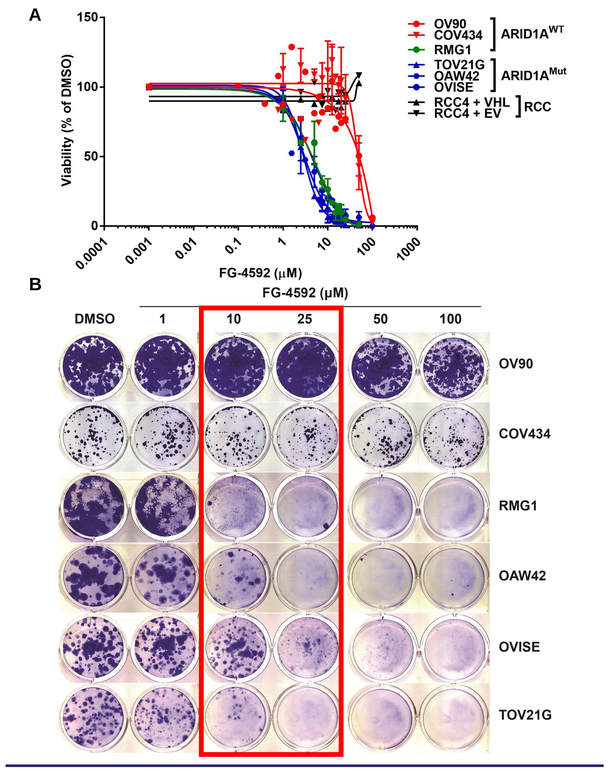

Figure 6: Pharmacological inhibition of the EGLN family recapitulates genetic EGLN1 perturbation data. ARID1A-WT ovarian cancer cell lines (OV90, COV434 and RMG1) and ARID1A-mutant ovarian cancer cell lines (OAW42, OVISE and TOV21G) were cultured for 2 weeks with increasing concentrations of a pan-EGLN inhibitor, FG-4592. The cells were then either counted with a Vi-Cell XR cell counter (A), or stained with crystal violet (B). Note: RMG1 is sensitive to the pan-EGLN inhibitor, FG-4592, which is inconsistent with the EGLN1-specific genetic perturbation data (Figure 3). RMG1 is an ARID1A^{WT} OCCC cell line that accumulates high levels of HIF1α when treated with a pan-EGLN inhibitor (Figure 5), which suggests a possible mechanism of action.

Sensitivity to EGLN inhibition is HIF-dependent

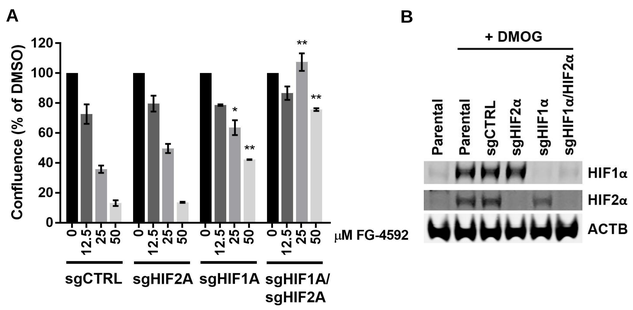

Figure 7: ARID1A-mutant ovarian cancer cell line sensitivity to pharmacological pan-EGLN inhibition is HIF-dependent. An ARID1A-mutant ovarian cancer cell line, TOV21G, was transduced with CRISPR sgRNAs either targeting HIF1A, HIF2A, both HIF1A and HIF2A, or a non-targeting sgRNA (sgCTRL). (A) The engineered cell lines were cultured for 10 days in the presence of the pan-EGLN inhibitor, FG-4592, at the indicated concentrations. Confluence data were acquired with an IncuCyte S3 system, and plotted relative to the DMSO control for each cell line. Data are represented as mean ± SEM of two replicates. Statistical data reflects t-test analysis performed on the indicated condition compared to the equivalent condition for sgCTRL. * refers to p<0.05, ** refers to p<0.01. (B) Immunoblot of cell lines used in (A). DMOG is a pan-EGLN inhibitor.

PI3Kα inhibitor is synergistic with EGLN inhibitor in ARID1A/PIK3CA-mutant ovarian cancer

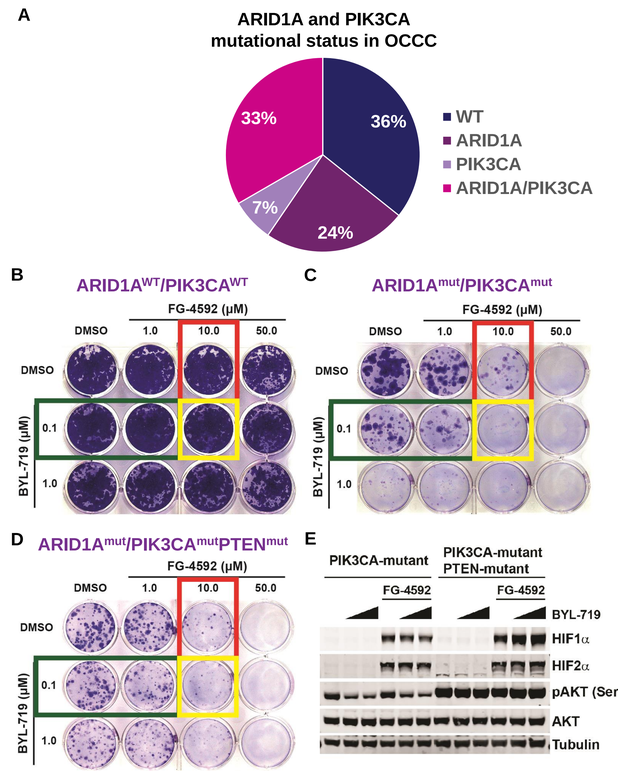

Figure 8: EGLN inhibition is synergistic with PI3Kα inhibition in ARID1A/PIK3CA-mutant ovarian cancer cell lines. (A) Incidence of PIK3CA and ARID1A mutations and co-mutations in ovarian clear cell carcinoma (Jones et al., 2010). (B-D) Crystal violet staining of ovarian cancer cell lines with the indicated ARID1A and PIK3CA mutational status cultured in the presence of the pan-EGLN inhibitor, FG-4592, and the PI3Kα-specific inhibitor, BYL-719, for 2 weeks. (E) Immunoblot of an ARID1A^{mutant}/PIK3CA^{mutant} and an ARID1A^{mutant}/PIK3CA^{mutant}/PTEN^{mutant} ovarian cancer cell line with increasing concentrations of BYL-719 and 50 μM FG-4592.

SUMMARY

- EGLN1 is a synthetic lethal target in ARID1A-mutant ovarian cancer
- ARID1A-mutant ovarian cancer cell lines are sensitive to a pan-EGLN inhibitor
- Sensitivity to pan-EGLN inhibitor is HIF-dependent
- EGLN inhibition is synergistic with PI3Kα inhibition
- Pharmacological inhibition of EGLN prolyl hydroxylases is well-tolerated in mammals
- EGLN inhibitors may be efficacious in the treatment of ARID1A-mutant OCCC

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