FBXO11 mediates PARP inhibitor resistance in small-cell lung cancer

Jiaqi Xiong[1,2], Mansi Aparnathi[1], Tony Yu[1,2], Lifang Song[1], Jonathan St-Germain[1], Brian Raught[1,2], Troy Ketela[1], Sami Ul Haq[1], Vivek Phillip[1], Benjamin Lok[1,2,3]

[1] Princess Margaret Cancer Centre, University Health Network, Toronto, ON , [2] Department of Medical Biophysics, University of Toronto, Toronto, ON , [3] Department of Radiation Oncology, University of Toronto, Toronto, ON

BACKGROUND

Small-cell lung cancer (SCLC) is an aggressive neuroendocrine tumor with limited treatment options and poor prognosis. PARP inhibitors (PARPi) showed promise in preclinical models of SCLC, yet its clinical application is challenging due to varying patient response, highlighting the need for predictive biomarkers. In a genome-wide CRISPR knockout (KO) screen, we identified the **SCF E3 ubiquitin ligase, FBXO11,** as a determinant of PARPi sensitivity in SCLC. Proteins of a pre-mRNA splicing complex (The XAB2 complex: **XAB2, AQR, ISY1**) identified as top interactors of FBXO11 in BioID-MS. These interactions suggest that FBXO11 may regulate alternative splicing, particularly genes in DNA damage response, offering a mechanistic link to PARPi resistance and highlighting its potential as a predictive biomarker in SCLC.

HYPOTHESIS

FBXO11 regulates PARPi sensitivity through its ubiquitination of XAB2 and regulation of the XAB2 splicing complex.

METHODS

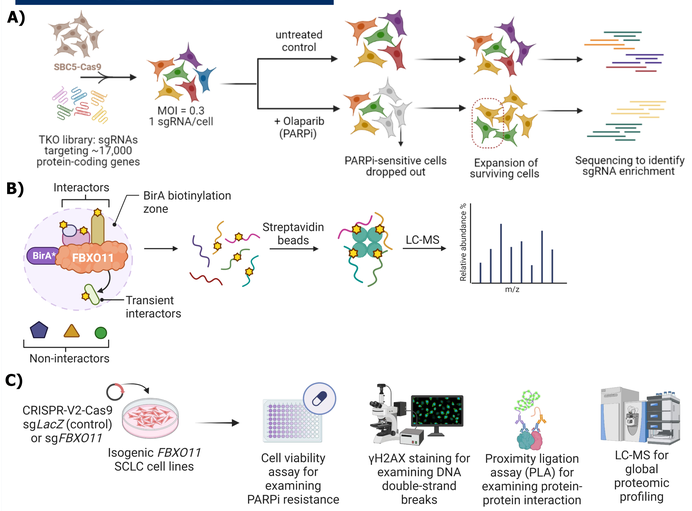

Figure 1. A) Schematic of a genome-wide CRISPR-Cas9 KO screen in the SBC5 SCLC cell line**.** SBC5-Cas9 cells are transduced with TKOv1 library with 5 μM olaparib as the selection pressure. Cells were collected at days 25 and 35 for genomic sequencing to determine sgRNA enrichment. B) Schematic of FBXO11 BioID. The BirA*-FBXO11 fusion protein is expressed in FlpIn HEK293 cells. Upon addition of biotin, proximal interactors of FBXO11 are biotinylated. Biotinylated proteins are enriched using streptavidin beads and identified via liquid chromatography – mass spectrometry (LC-MS). C) Workflow of phenotypic and functional analyses of isogenic *FBXO11* cells.

RESULTS

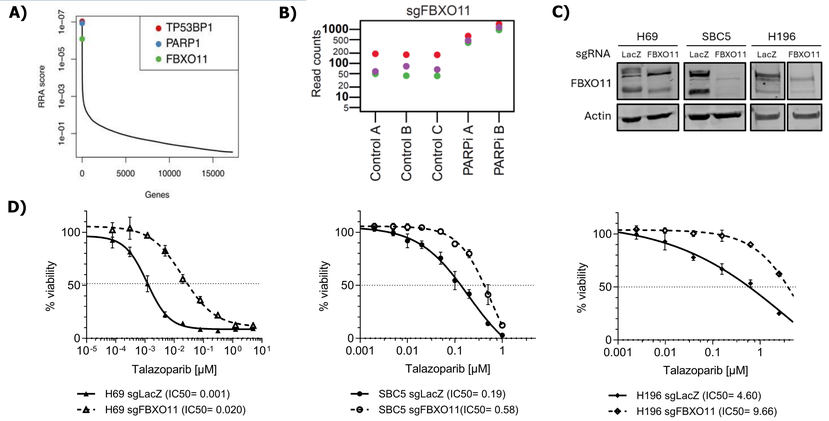

Figure 2. Genome-wide CRISPR KO screen in SBC5 SCLC cells identified loss of *FBXO11* as a putative determinant of PARPi resistance. A) Robust Ranking Algorithm (RRA) with top 3 sgRNA hits (*TP53BP1*, *PARP1*, and *FBXO11*). B) Read counts of independent sgRNAs against *FBXO11* in control and PARPi treated groups. C) Western blot of isogenic *FBXO11* SCLC cell lines with labeled sgRNA and antibodies. D) 7-day cell viability demonstrating talazoparib resistance in 3 pairs of isogenic *FBXO11* SCLC cell lines (H69, SBC5, H196). IC50 = half-maximal inhibitory concentration.

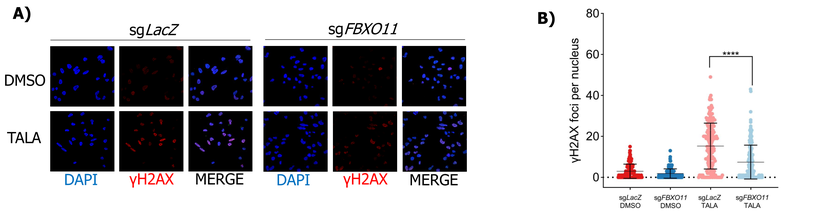

Figure 3. *FBXO11*-KO SBC5 cells had significantly reduced γH2AX foci formation following talazoparib treatment (n=3). A) Representative images of γH2AX foci in isogenic *FBXO11* SBC5 cell lines with either DMSO or 24h talazoparib (100 nM) treatment. TALA = talazoparib. B) Quantification of average γH2AX foci per nucleus ($p < 0.0001$).

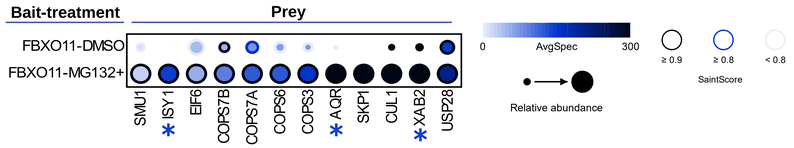

Figure 4. FBXO11 BioID identified proteins ISY1, AQR, and XAB2 as putative interactors. Proteins ISY1, AQR, and XAB2, components of the XAB2 complex appeared as high-confidence interactors of FBXO11 in BioID after MG132 (a proteosome inhibitor) treatment. Notably, XAB2 is a high-confidence interactor of FBXO11 even without the presence of MG132. Saint Score = **S**ignificance **A**nalysis of **INT**eractome, indicates the probability of a true interaction.

RESULTS

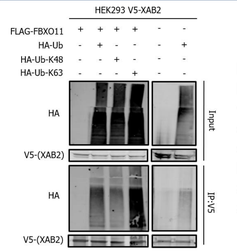

Figure 5. FBXO11 promotes K63-linked ubiquitination of XAB2. HEK293 cells stably expressing V5-tagged XAB2 were transiently transfected with FLAG-tagged FBXO11 or an empty vector control. In parallel, cells were co-transfected with HA-tagged ubiquitin constructs – either wild type (HA-Ub) or ubiquitin mutants with either lysine 48 (HA-Ub-K48) or lysine 63 (HA-Ub-K63) intact. Denaturing immunoprecipitation of V5-XAB2 was done using V5 antibody affinity beads, and the enrichment of ubiquitination was analyzed to assess the role of FBXO11 in promoting K63-linked ubiquitination.

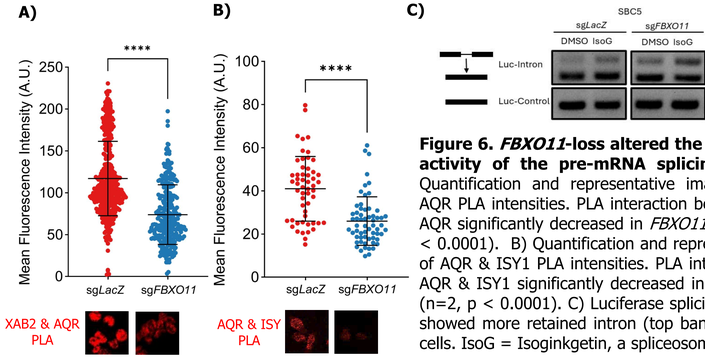

Figure 6. *FBXO11*-loss altered the interaction and activity of the pre-mRNA splicing complex. A) Quantification and representative images of XAB2 & AQR PLA intensities. PLA interaction between XAB2 and AQR significantly decreased in *FBXO11*-KO cells (n=3, $p < 0.0001$). B) Quantification and representative images of AQR & ISY1 PLA intensities. PLA interaction between AQR & ISY1 significantly decreased in *FBXO11*-KO cells (n=2, $p < 0.0001$). C) Luciferase splicing reporter assay showed more retained intron (top band) in *FBXO11*-KO cells. IsoG = Isoginkgetin, a spliceosome inhibitor.

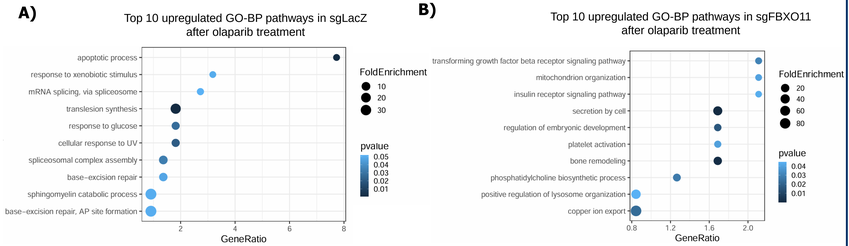

Figure 7. Isogenic *FBXO11* cells exhibited differentially upregulated pathways after olaparib treatment. A) Proteins involved in apoptotic pathways, mRNA splicing, and spliceosomal complex assembly were upregulated in sg*LacZ* cells. B) Proteins involved in pathways that facilitated cellular growth were upregulated in sg*FBXO11* cells.

Conclusion & Future Direction

- *FBXO11*-loss is a novel determinant of PARPi resistance in SCLC, potentially through its regulation of the pre-mRNA splicing complex, the XAB2 complex.
- RNA sequencing of isogenic *FBXO11* cells will be conducted to examine alternatively spliced genes involved in DNA damage response and apoptosis.