Cells 2025 Conference
The 3rd International Online Conference on Cells
25–27 March 2025 | Online
MDPI

Unravelling the role of AP-1 transcription factor in DNA damage signaling and response (DDR), platinum and PARP inhibitor resistance in ovarian cancers

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INTRODUCTION & AIM

Introduction: Hypoxia is a feature of high grade serous ovarian cancer (HGSOC) microenvironment and contributes to platinum and PARP inhibitor resistance. Hypoxia induces activator protein-1 (AP-1) transcription factor (TF) activity leading to sustained proliferation, invasion, metastasis and angiogenesis. The role of AP-1 in DNA damage signalling and repair (DDR) and platinum/PARP resistance is unclear in HGSOC.

Hypothesis: 1) AP1 transcription factor is involved in the regulation of DNA repair genes. 2) AP1 upregulation is involved in the development of resistance to DNA repair blockade. 3) AP1 blockade could be a potential drug target in HGSOC.

METHOD

A) Expression of FOSL2 were investigated in 331 clinical epithelial ovarian cancers. B) Evaluation of JUNB, FOSL2 depletion (by CRISPR/Cas-9 knock out) in PEO1, PEO1R ovarian cancer cell lines and evaluate cellular phenotype and response to the cisplatin/PARP therapy. C) Study the effect of FOSL2 and JUNB KO in ovarian tumour spheres.
D) Compare the DNA repair genes levels between control and KO cells using profiler PCR arrays.

RESULTS & DISCUSSION

FOSL2 expression was significantly associated with poor Progression free survival (PFS) and overall survival (OS)

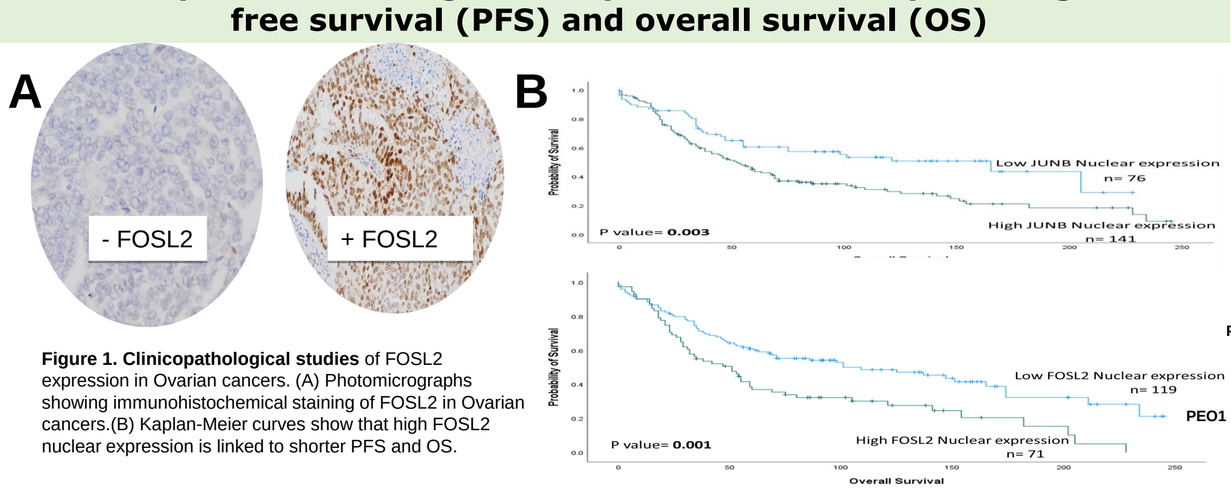

Figure 1. Clinicopathological studies of FOSL2 expression in Ovarian cancers. (A) Photomicrographs showing immunohistochemical staining of FOSL2 in Ovarian cancers.(B) Kaplan-Meier curves show that high FOSL2 nuclear expression is linked to shorter PFS and OS.

FOSL2 KO hypoxic ovarian cancer cells were sensitive to cisplatin treatment compared to controls

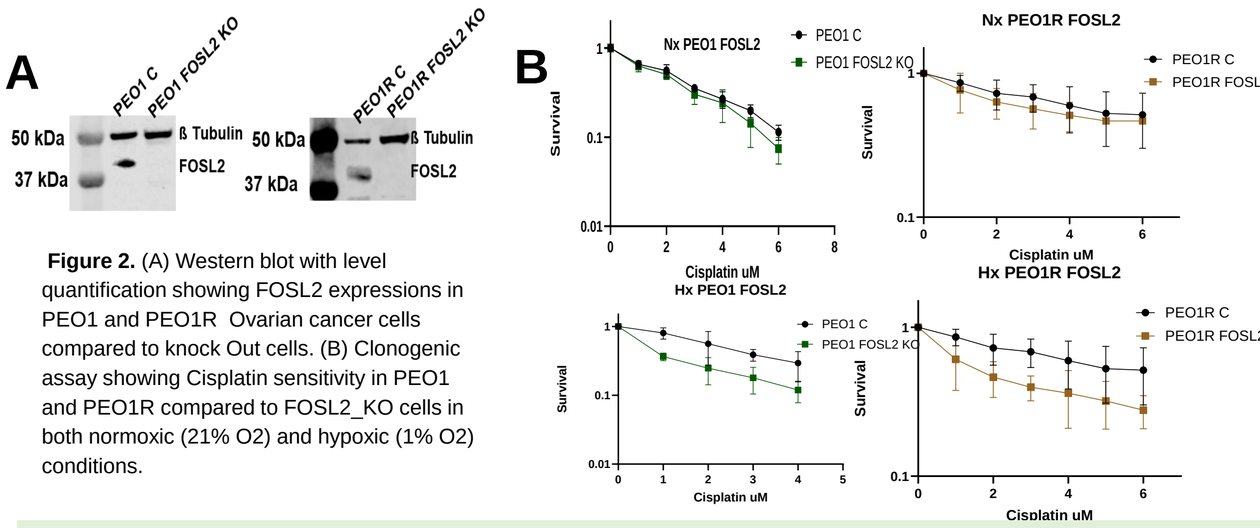

Figure 2. (A) Western blot with level quantification showing FOSL2 expressions in PEO1 and PEO1R Ovarian cancer cells compared to knock Out cells. (B) Clonogenic assay showing Cisplatin sensitivity in PEO1 and PEO1R compared to FOSL2_KO cells in both normoxic (21% O2) and hypoxic (1% O2) conditions.

Increased sensitivity to cisplatin and Olaparib were associated with DNA double strand break accumulation in FOSL2 KO ovarian cancer cells

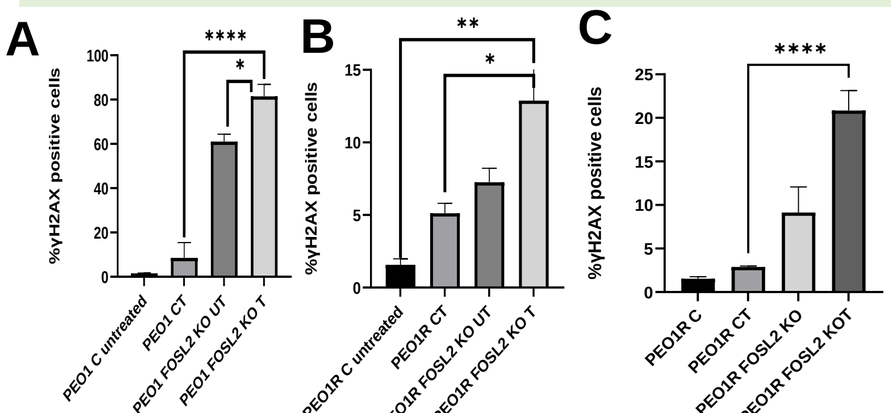

Figure 3. Data showing the percentage of H2AX-positive cells in control and knock out cells, untreated or treated with cisplatin/PARP and quantification of % H2AX positive cells by flow cytometry. (A) PEO1_C and PEO1_FOSL2_KO treated with 5uM Cisplatin. (B) PEO1R_C and PEO1R_FOSL2_KO treated with 5uM Cisplatin. (C) PEO1R_C and PEO1R_FOSL2_KO treated with 6uM Olaparib.

G2M-phase cell cycle arrest in FOSL2 KO ovarian cancer cells

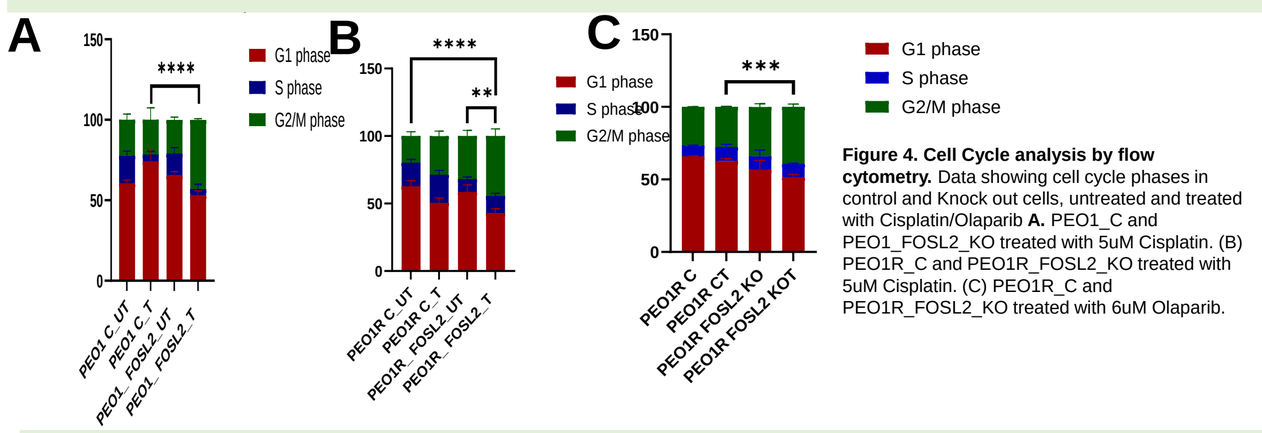

Figure 4. Cell Cycle analysis by flow cytometry. Data showing cell cycle phases in control and Knock out cells, untreated and treated with Cisplatin/Olaparib **A.** PEO1_C and PEO1_FOSL2_KO treated with 5uM Cisplatin. (B) PEO1R_C and PEO1R_FOSL2_KO treated with 5uM Cisplatin. (C) PEO1R_C and PEO1R_FOSL2_KO treated with 6uM Olaparib.

Increased apoptosis in FOSL2 KO ovarian cancer cells

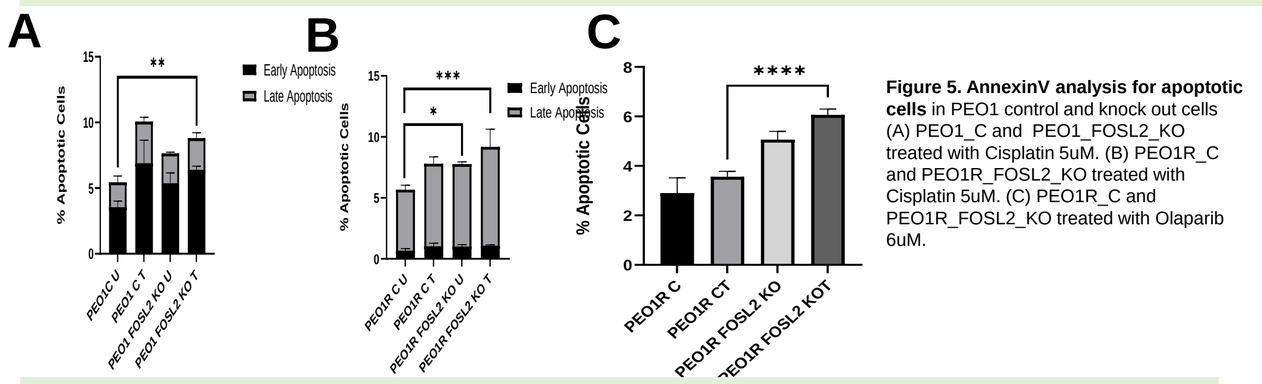

Figure 5. AnnexinV analysis for apoptotic cells in PEO1 control and knock out cells (A) PEO1_C and PEO1_FOSL2_KO treated with Cisplatin 5uM. (B) PEO1R_C and PEO1R_FOSL2_KO treated with Cisplatin 5uM. (C) PEO1R_C and PEO1R_FOSL2_KO treated with Olaparib 6uM.

Decrease in spheroid size and increased dead cells in FOSL2_KO cells

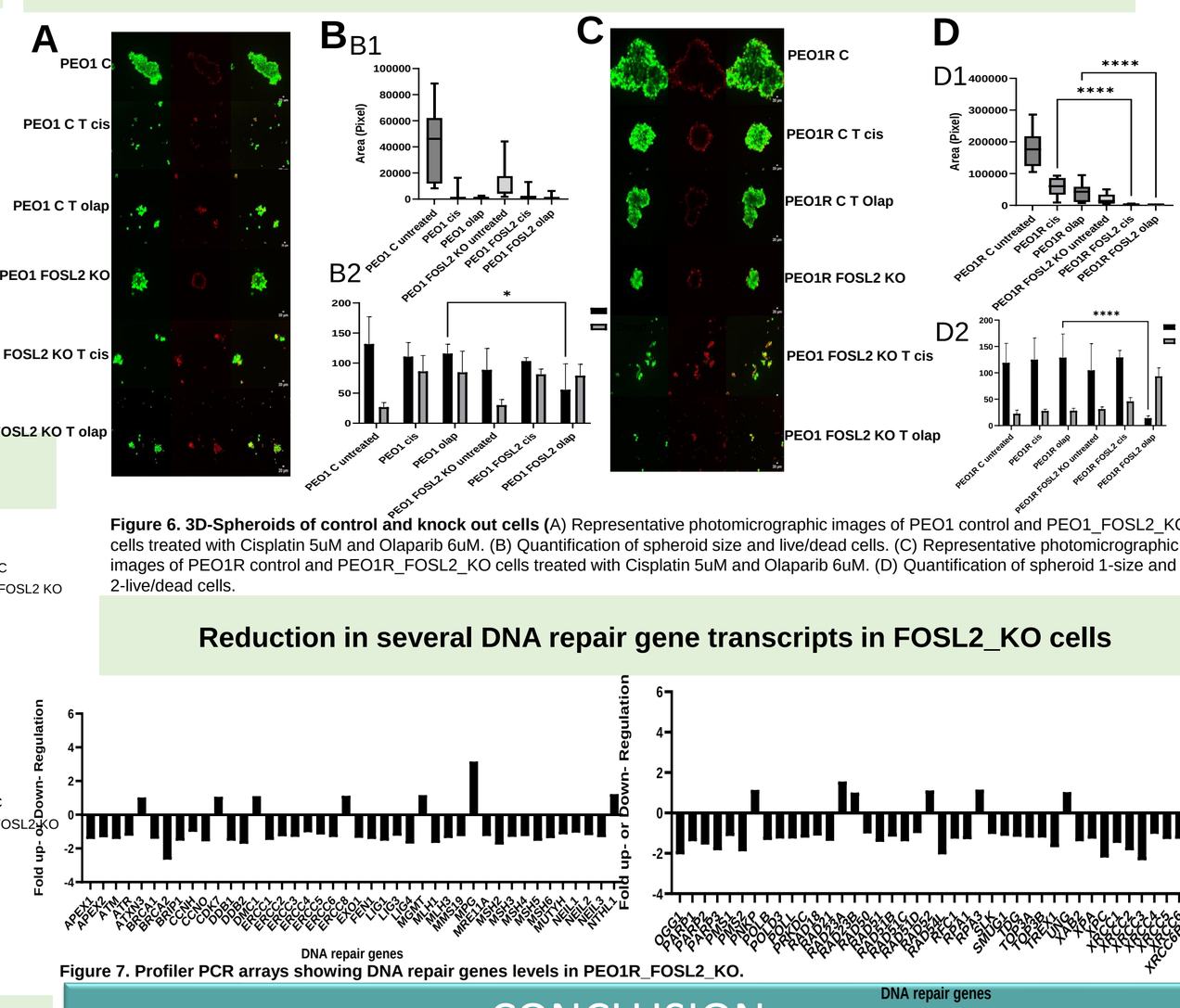

Figure 6. 3D-Spheroids of control and knock out cells (A) Representative photomicrographic images of PEO1 control and PEO1_FOSL2_KO cells treated with Cisplatin 5uM and Olaparib 6uM. (B) Quantification of spheroid size and live/dead cells. (C) Representative photomicrographic images of PEO1R control and PEO1R_FOSL2_KO cells treated with Cisplatin 5uM and Olaparib 6uM. (D) Quantification of spheroid 1-size and 2-live/dead cells.

Reduction in several DNA repair gene transcripts in FOSL2_KO cells

Figure 7. Profiler PCR arrays showing DNA repair genes levels in PEO1R_FOSL2_KO.

CONCLUSION

Our data provides evidence that FOSL2 may operate at the hypoxia-DDR interface in HGSOC. FOSL2 not only has predictive and prognostic significance but could also be attractive anti-cancer targets including in platinum/PARP resistant HGSOC.

Shaik, B., et al. (2021). "An overview of ovarian cancer: molecular processes involved and development of target-based chemotherapeutics." Current topics in medicinal chemistry 21(4): 329-346.
Wu, Z., et al. (2021). "AP-1 family transcription factors: a diverse family of proteins that regulate varied cellular activities in classical hodgkin lymphoma and ALK+ ALCL." Experimental hematology & oncology 10(1): 1-12.