The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin

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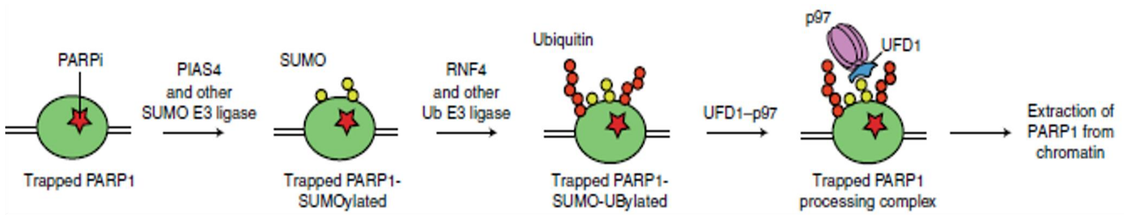
INTRODUCTION

Poly (ADP-ribose) polymerase 1 (PARP1) is a crucial enzyme in the DNA damage response which facilitates repair through poly(ADP-ribosyl)ation (PARylation)[1]. However, PARP inhibitors (PARPi) causes persistent trapping of PARP1 on chromatin which leads to cytotoxicity, a mechanism that cancer therapies exploit to target homologous recombination-deficient tumors [2]. The ubiquitin-dependent ATPase p97 (also known as VCP) is a molecular chaperone vital for protein homeostasis, which includes the extraction of ubiquitinated proteins from chromatin. Recent studies have shown that p97 is able to resolve trapped PARP1 complexes using its ATPase activity, maintaining genomic stability and preventing cell death [3]. This research investigates the molecular mechanisms involved in p97-mediated removal of PARP1, highlighting its therapeutic implications in modulating PARPi sensitivity.



METHODS AND MATERIALS

Human osteosarcoma U2OS cells and were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin- streptomycin. Olaparib was used to induce PARP1 trapping, while methyl methanesulfonate (MMS) was used to create DNA lesions[2]. A sequential salt extraction protocol was used to isolate chromatin-bound proteins and to separate chromatin-associated complexes from soluble nuclear proteins[4]. To assess PARP1 levels, ubiquitination, and p97 association using specific antibodies, immunoblotting was performed. Ubiquitination assays were conducted in vitro with recombinant PARP1, ubiquitin, E1/E2 enzymes, and p97, and in vivo by immunoprecipitating PARP1 from cell lysates followed by anti-ubiquitin immunoblotting[1]. Immunofluorescence microscopy was also used to visualize PARP1 trapping, DNA damage markers (YH2AX), and colocalization with p97 using confocal imaging. By using a malachite green-based phosphate detection assay to measure ATP hydrolysis, p97 ATPase activity was quantified. Using all these methods, the role of p97 in resolving trapped PARP1 complexes and maintaining genomic stability was elucidated.

RESULTS

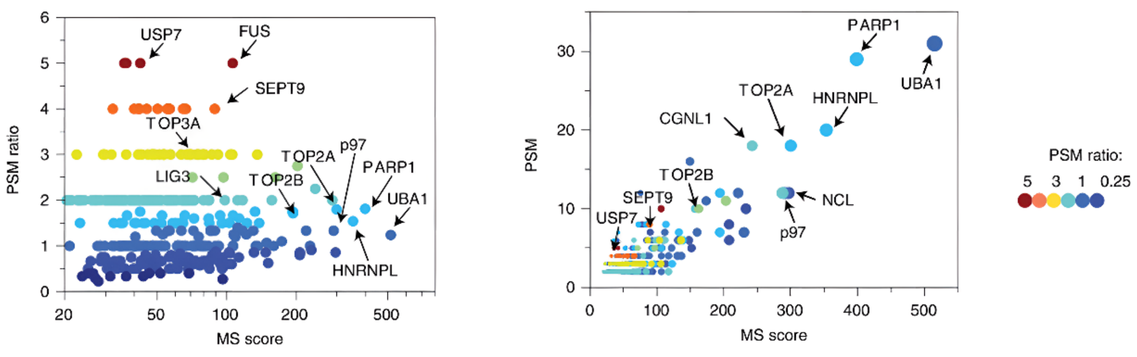
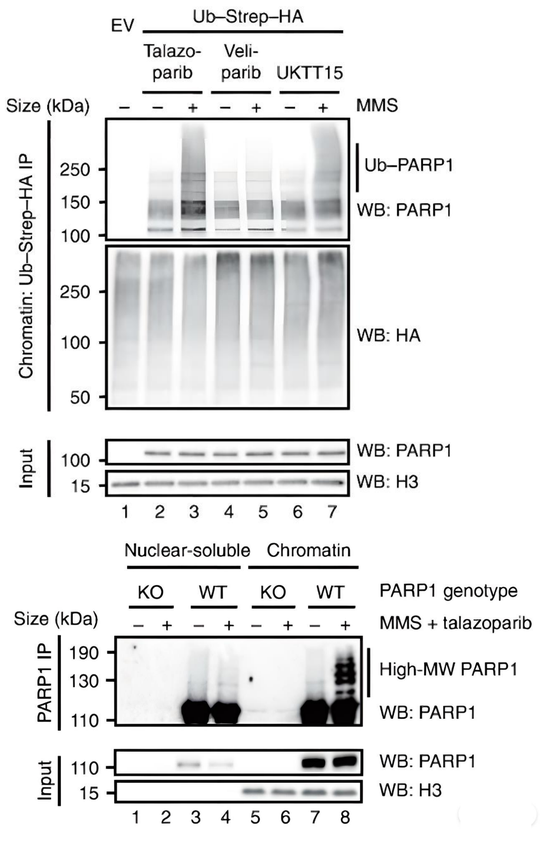


Figure 1. Mass Spectrometry (MS) and Peptide Spectrum Match (PSM) ratios showing p97- PARP1 interaction that is enriched under PARP1-trapping conditions for PARP1WT–Apex2–eGFP proximity labelling and p97 is among the most abundant proteins identified in the PARP1WT–Apex2–eGFP proximity labelling.



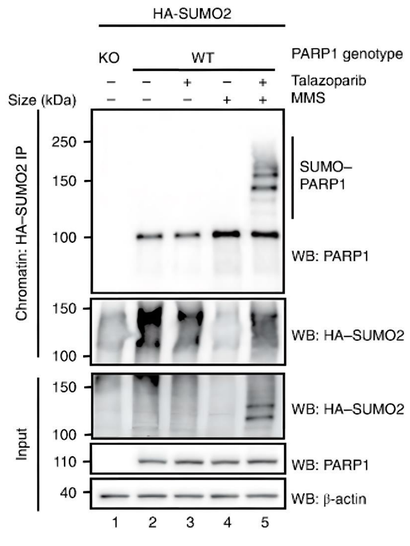


Figure 2. Western Blots showing evidence that PARP1 trapping leads to PARP1 ubiquitylation and Trapped PARP1 is SUMOylated. PARP1-trapping conditions elicit high-molecular-weight forms of PARP1 in the chromatin fraction.

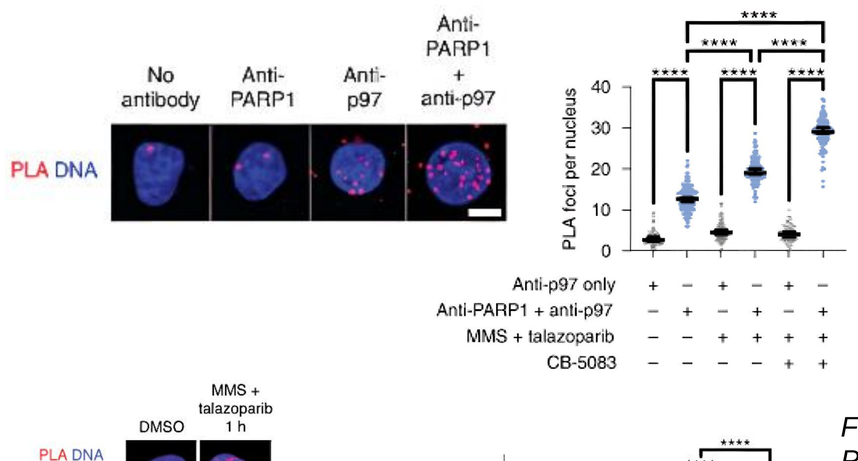


Figure 3. Images of a Proximity Ligation Assay (PLA) for endogenous PARP1 and p97 in CAL51 cells and number of PLA foci.

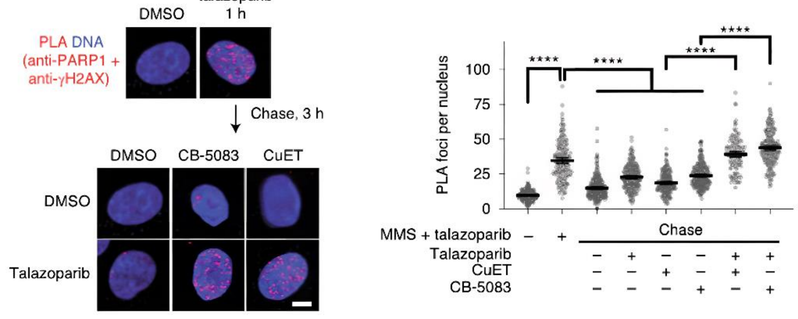


Figure 4. Images of PLA and number of PARP1–γH2AX foci in the trap–chase experiment. PARP1–γH2AX PLA foci persist in cells chased in PARPi plus p97 inhibitors CB-5083 and CuET.

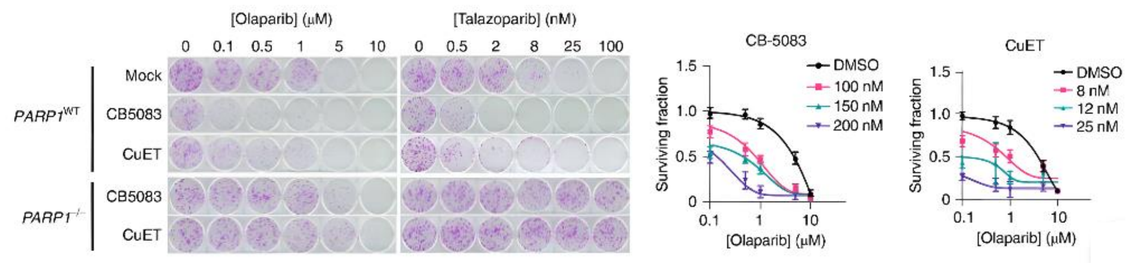


Figure 5. Viability assay and drug response curves showing combination of PARP inhibitors (PARPi) with p97 inhibitors enhances cytotoxicity.

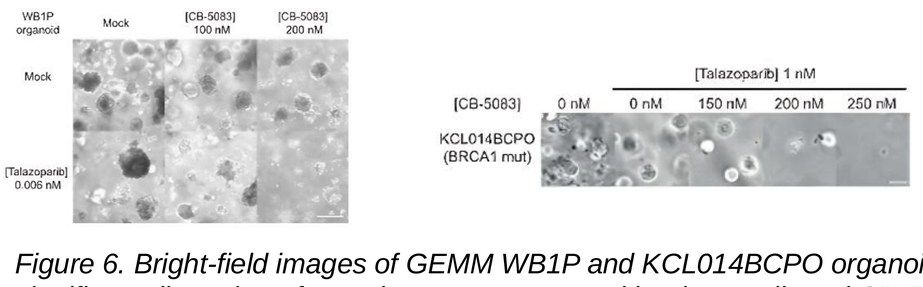


Figure 6. Bright-field images of GEMM WB1P and KCL014BCPO organoids showing significant disruption of growth upon treatment with talazoparib and CB-5083 combination.

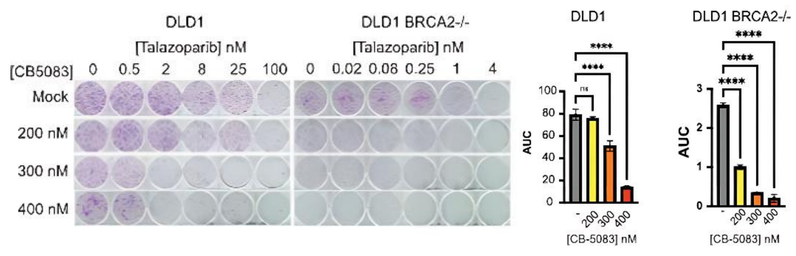


Figure 7. Colony formation assays and AUC analysis showing the synergistic cytotoxic effects of combining talazoparib (PARPi) and CB-5083 (p97 inhibitor), in BRCA2 −/− cells.

KEY FINDINGS

- Trapped PARP1 is processed through SUMOylation (PIAS4) and ubiquitylation (RNF4), followed by removal via p97.
- Interruption in this cascade enhances PARPi sensitivity, linking the pathway directly to therapeutic outcomes.
- PARPi-generated DNA lesions and trapped TOP1-cleavage complexes share a common processing pathway involving SUMOylation, ubiquitylation, and p97 modification, suggesting a shared sensing and repair machinery that may broadly influence DNA metabolism.

FURTHER QUESTIONS

- Do other E3 ligases influence the balance of SUMOylation and ubiquitylation in this process?
- How does UFD1 recruit p97 independently of NPL4?
- What determines the decision between recycling and degradation for PARP1?

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