



Jefferson. A Novel PARP Inhibitor Resistance Mechanism Mediated by the RNA-Binding Protein HuR

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer-related deaths in the United States, and ranks as the third most common cancer associated with BRCA mutations, not including Fanconi Anemia gene mutations such as PALB2. Current combinations of chemotherapeutics have significant toxicities and only minimally extend overall patient survival. Poly-ADP ribose polymerase (PARP) inhibitors (PARPi), which target the DNA damage repair (DDR) pathway, have delivered promising preclinical and clinical results. Although synthetic lethality conferred by PARPi is a promising approach, still "selected" patients with DNA repair-deficient tumors (e.g., BRCA2 mutated) that initially respond to such molecular-targeted therapies ultimately develop resistance. Therefore, optimizing PARPi therapy requires a thorough understanding of associated drug resistance mechanisms.

Here, we demonstrate that the mRNA-binding protein, HuR supports a novel resistance mechanism via post-transcriptional regulation of select mRNA cargo. Predominantly expressed in the nucleus, HuR translocates to the cytoplasm upon tumor-associated stress (e.g. cytotoxic agents). Cytoplasmic HuR binds and stabilizes unique pro-survival transcripts resulting in resistance to a harsh tumor microenvironment (Figure 1).

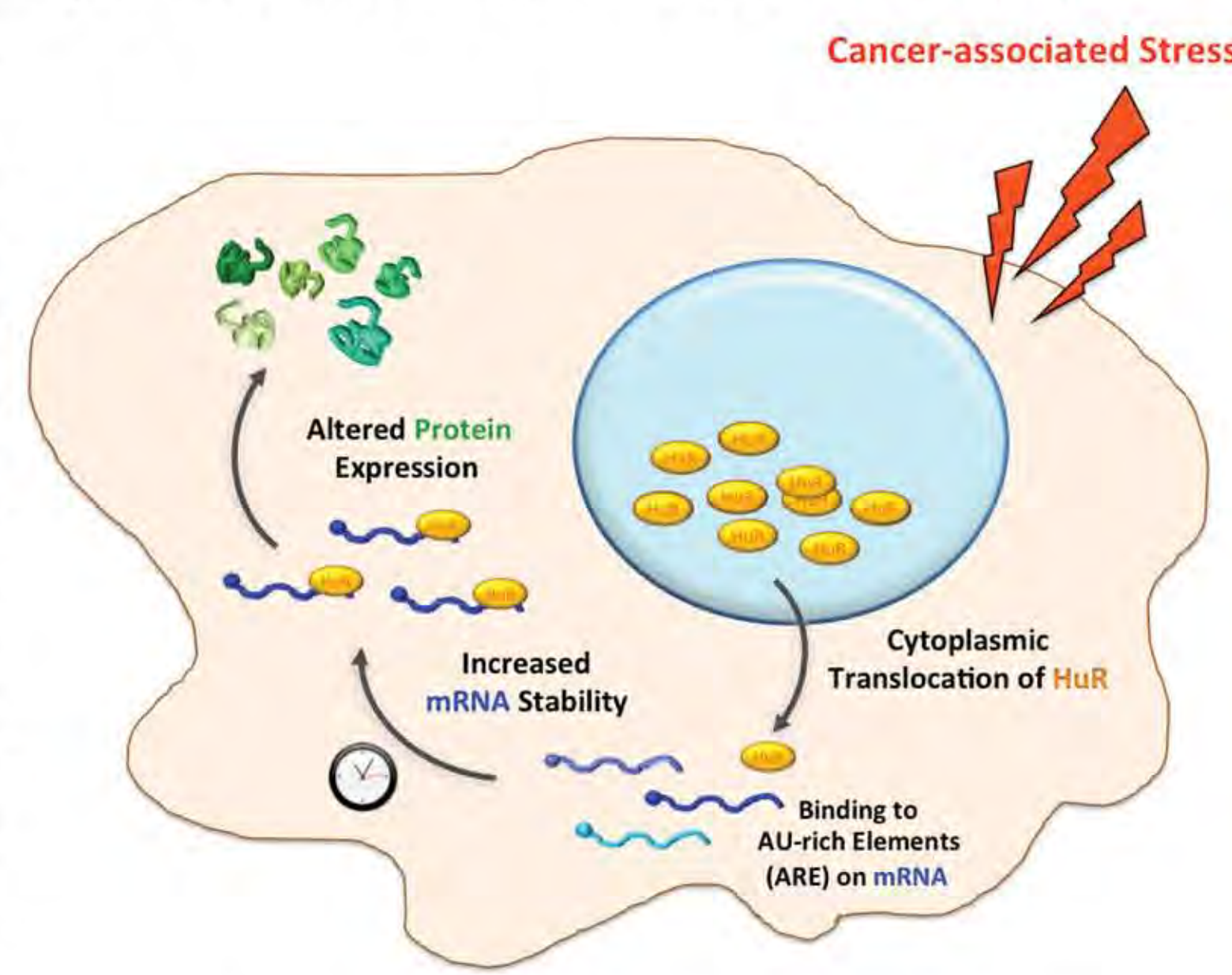


Figure 1. Post-transcriptional regulation mediated by HuR.

Through immunofluorescence and western blot of fractionated lysates, we demonstrate that the PARP inhibitors ABT-888, Olaparib, and Rucaparib induced cytoplasmic HuR localization. Conversely, pre-treatment with MS-444 (Novartis), an established small molecule inhibitor of HuR, abrogated its nuclear export induced by PARPi treatment. Consistent with these findings, siRNA-mediated knockdown of HuR enhanced sensitivity to the cytotoxic effects of PARP inhibition, whereas ectopic HuR overexpression promoted resistance. To further validate the role of HuR in cell survival, we evaluated anchorage-independent growth of PDA cells transfected with siRNA against HuR and subjected to PARPi treatment. To this extent, the growth-inhibitory effects of PARPi treatment were significantly potentiated upon HuR silencing. Also, silencing of HuR enhances PARPi-induced DNA damage assessed by γ H2AX foci formation. Taken together, these results demonstrate that HuR imposes a significant barrier to PARPi therapy by orchestrating a strong chemoresistance mechanism. Thus, we provide evidence that HuR inhibition can optimize PARPi-based therapies. Ongoing work will determine key HuR targets responsible for effecting PARPi efficacy in pancreatic cancer cells.

PARPi induces cytoplasmic translocation of HuR

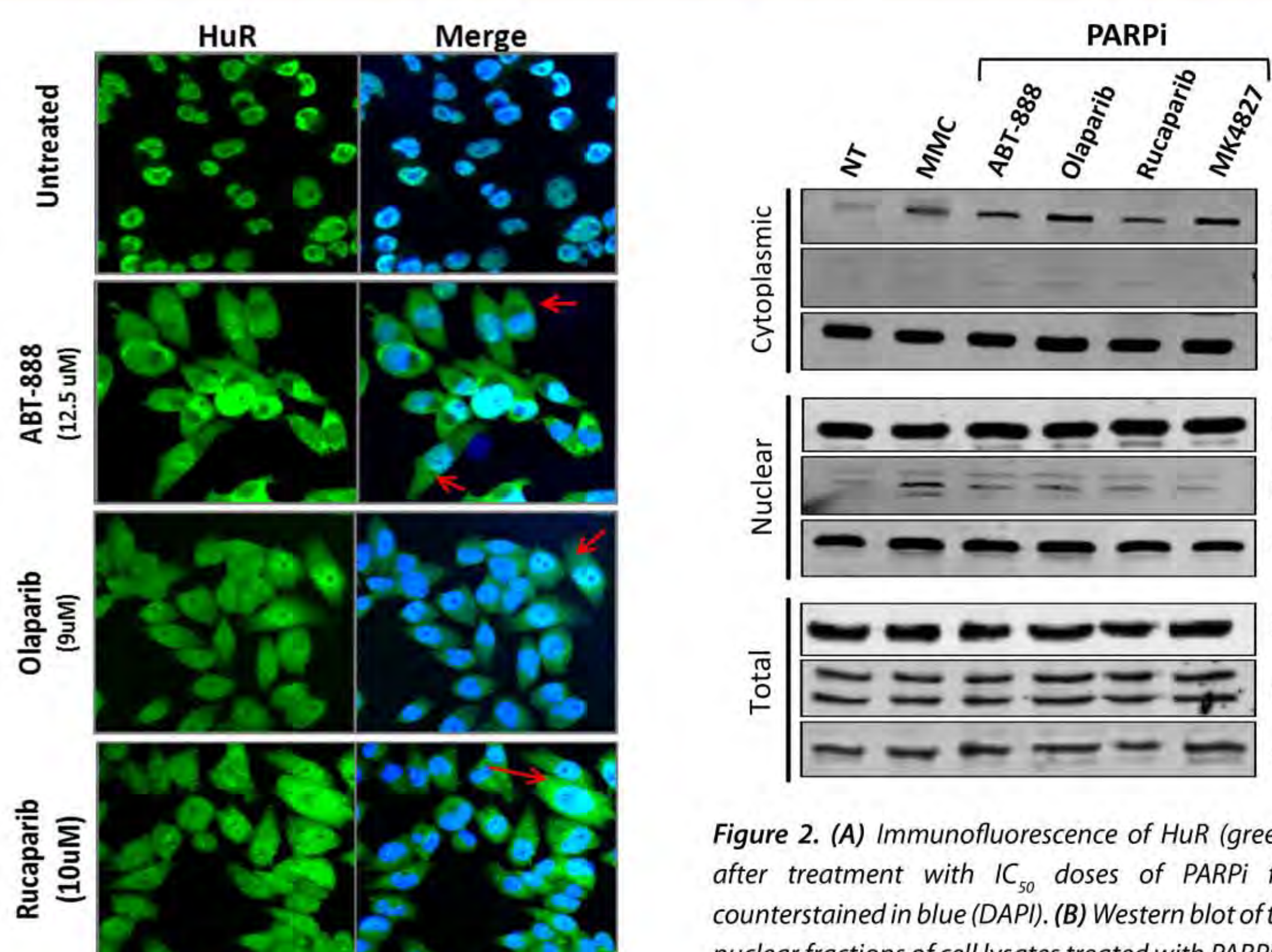


Figure 2. (A) Immunofluorescence of HuR (green) in MiaPaCa2 cells after treatment with IC_{50} doses of PARPi for 12h. Nuclei are counterstained in blue (DAPI). (B) Western blot of total, cytoplasmic and nuclear fractions of cell lysates treated with PARPi.

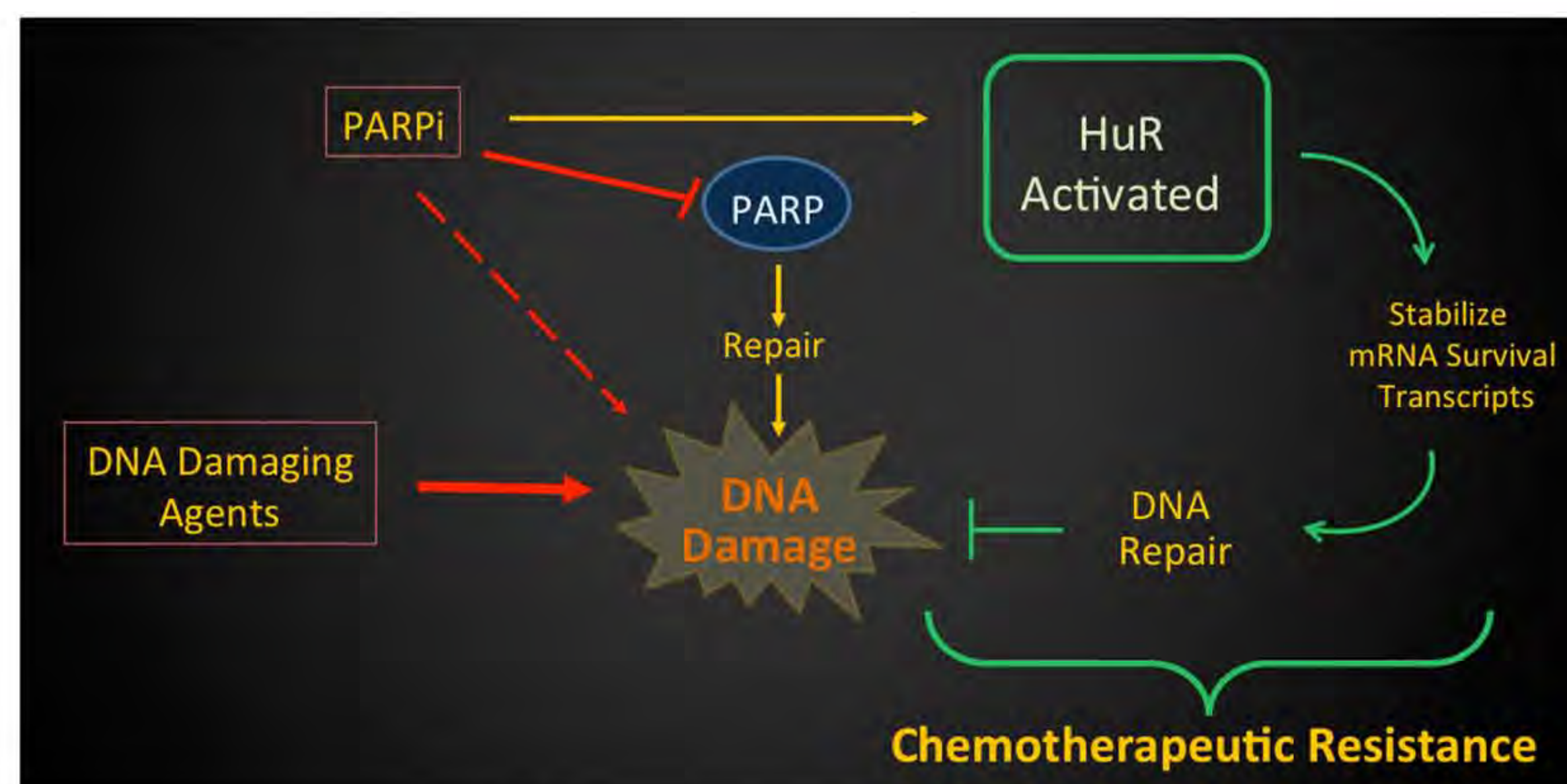


Figure 3. Proposed model: In response to PARPi therapy, HuR binds to and stabilizes select survival transcripts, and thereby supports a unique drug resistance mechanism.

Silencing of HuR increases sensitivity to PARPi in PDA cells

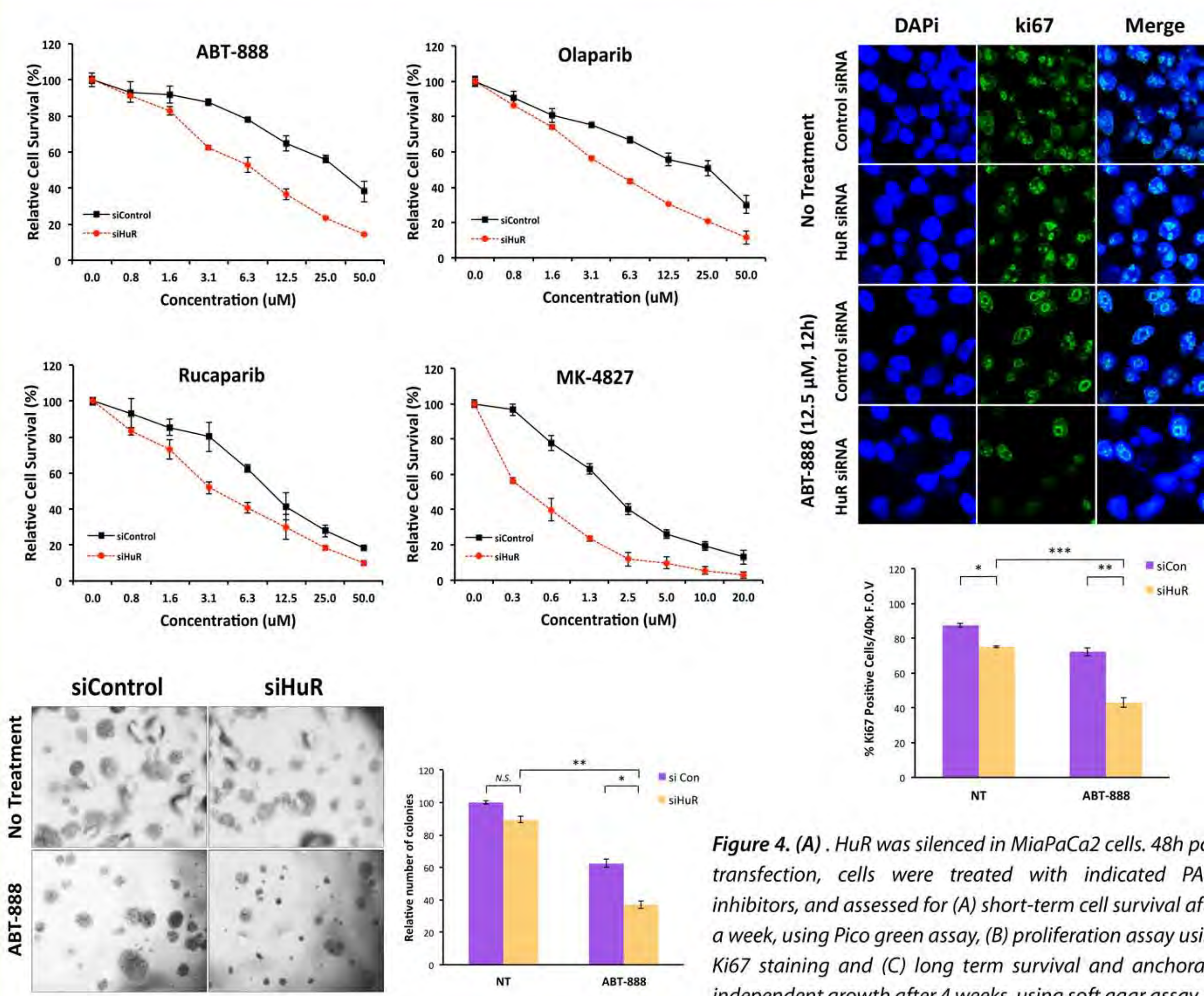


Figure 4. (A) HuR was silenced in MiaPaCa2 cells. 48h post transfection, cells were treated with indicated PARP inhibitors, and assessed for (A) short-term cell survival after a week, using Pico green assay, (B) proliferation assay using Ki67 staining and (C) long term survival and anchorage independent growth after 4 weeks, using soft agar assay.

Silencing of HuR enhances PARPi-induced DNA damage

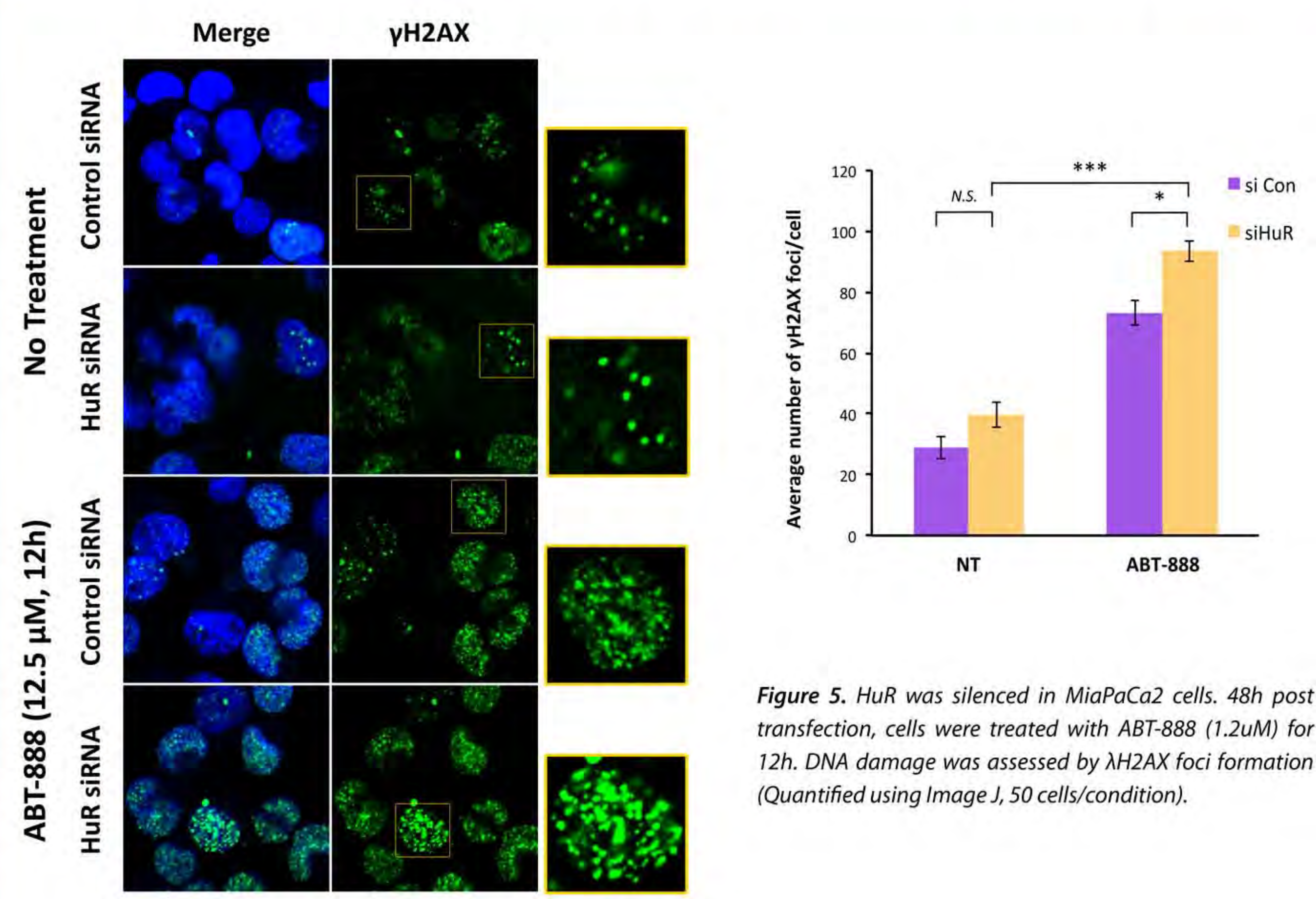


Figure 5. HuR was silenced in MiaPaCa2 cells. 48h post transfection, cells were treated with ABT-888 (1.2uM) for 12h. DNA damage was assessed by γ H2AX foci formation (Quantified using Image J, 50 cells/condition).

HuR inhibitor, MS-444 synergizes with PARPi

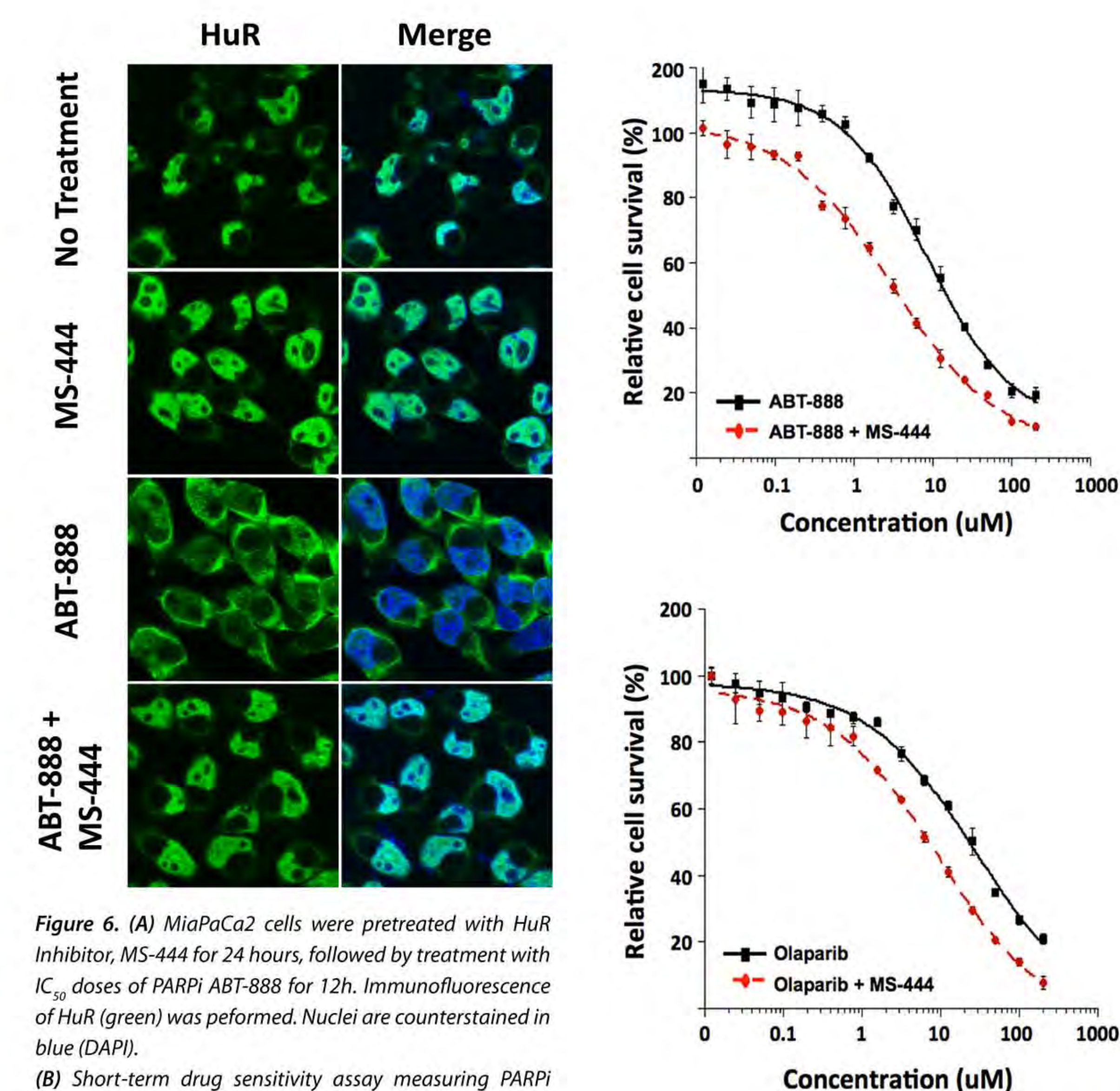


Figure 6. (A) MiaPaCa2 cells were pretreated with HuR inhibitor, MS-444 for 24 hours, followed by treatment with IC_{50} doses of PARPi ABT-888 for 12h. Immunofluorescence of HuR (green) was performed. Nuclei are counterstained in blue (DAPI). (B) Short-term drug sensitivity assay measuring PARPi (ABT-888, Olaparib) efficacy in MiaPaCa2 cells, in the presence and absence of MS-444.

Summary

We propose that HuR mediates a novel DNA damage response mechanism in PDA cells. Evaluation of this resistance mechanism holds promise for improving PARPi efficacy.

1. PARP inhibitors (Veliparib, Olaparib, Rucaparib, Iniparib) induce cytoplasmic translocation of HuR (Fig. 2).
2. HuR enhances long term and short term cell survival, proliferation and anchorage independent growth (Fig. 4).
3. DNA damage induced by PARP inhibitor ABT-888, is potentiated via RNAi-induced silencing of HuR (Fig. 5).
4. Small molecule HuR inhibitor MS-444, prevents HuR translocation and synergizes to potentiate PARPi sensitivity (Fig. 6).

Future Directions

In an effort to inhibit drug resistance, we propose to incorporate HuR inhibition in combination with PARPi-based therapy for pancreatic cancer.

1. Recognize shared and distinct HuR targets – systematic interrogation of HuR-transcripts from RIP-Seq of PARPi treated PDA cells.
2. Validate the proposed mechanism *ex vivo* – use clinical samples from an ongoing prospective Phase I/II trial of PARP inhibitors in patients with metastatic pancreatic cancer.
3. Analyze expression of identified HuR-targets to screen for potential predictive and prognostic biomarkers.
4. Determine the significance of this resistance mechanism in regards to BRCA/FA deficiency.

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