Identification of a novel role for PSPC1 in the Fanconi Anaemia repair pathway

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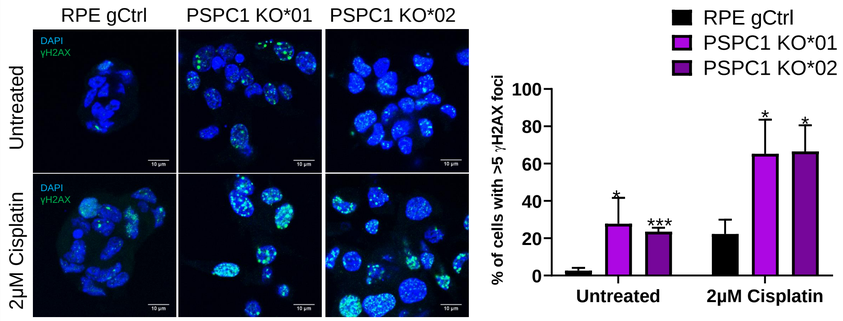
Abstract

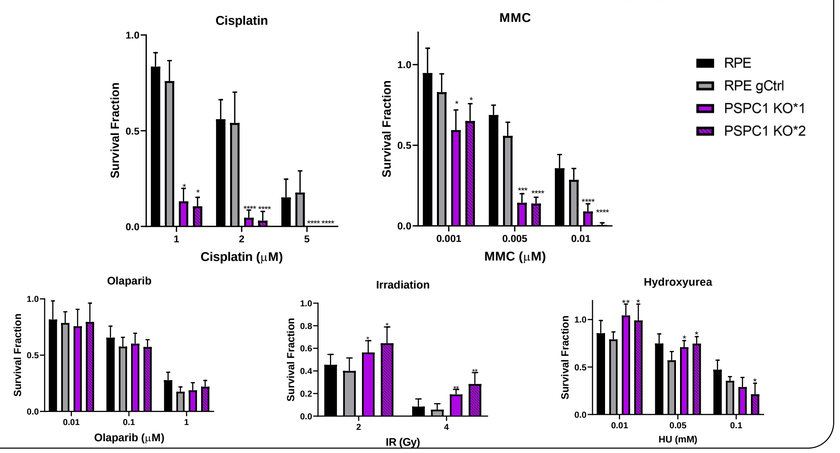
PSPC1 is a member of the Drosophila behaviour/human splicing protein family (DBHS), which were previously found to have a role in DNA repair, however, its precise functions in the DNA damage response (DDR) is unclear. To further understand the role of PSPC1 in response to DNA damage, we used CRISPR-Cas9 to knockout PSPC1 in RPE1 cells and treated cells with several drugs which induce DNA damage including cisplatin, mitomycin C (MMC), hydroxyurea (HU), Olaparib and irradiation. Interestingly, we observed that loss of PSPC1 promotes sensitivity to the DNA interstrand crosslinking agents, Cisplatin and MMC, but not upon treatment with HU, olaparib or irradiation. Given that cells with a deficiency in the Fanconi Anaemia (FA) pathway are more sensitive to DNA interstrand crosslinking agents, we next investigated the role of PSPC1 in the FA pathway. FA pathway is a DNA repair mechanism to resolve the DNA interstrand crosslinks. We observed the expression of FANCD2, which is one of the crucial protein in FA pathway, is affected by depleting PSPC1. This suggests PSPC1 has a key role in the regulation of the FA pathway.

RESULTS

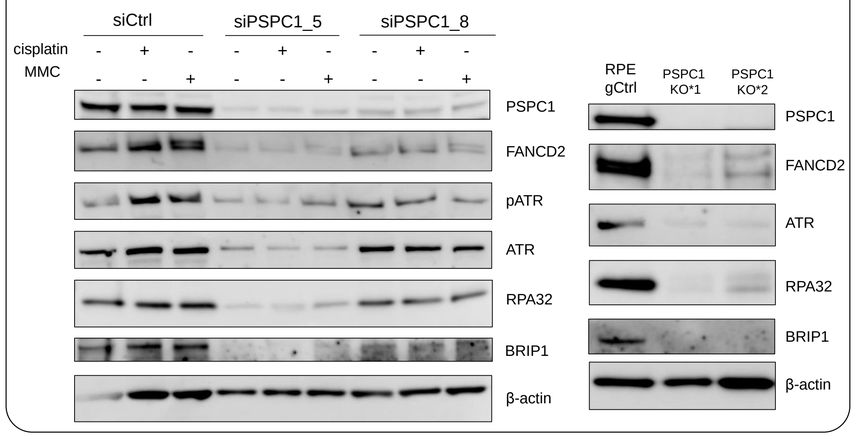
1. Increased DNA double strand breaks upon cisplatin treatment in PSPC1 knockout cells



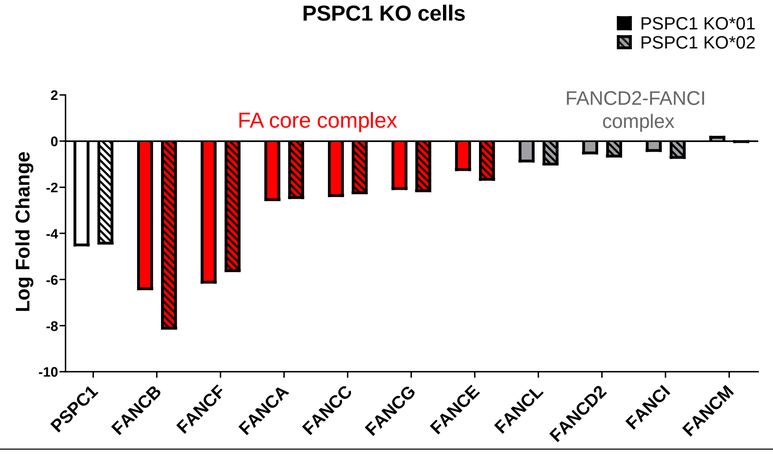
2. PSPC1 knockout cells are sensitive to ICL-inducing agents



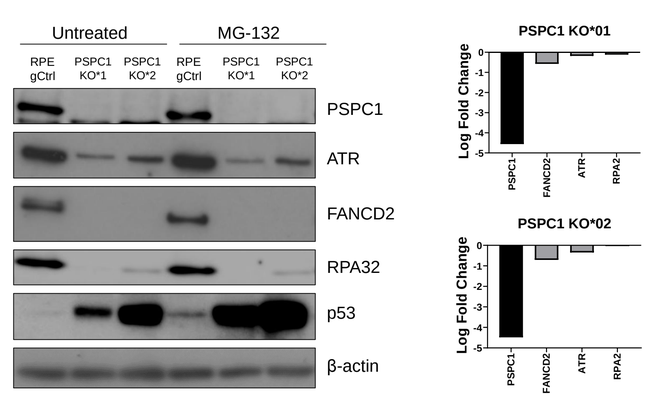
3. FANCD2, ATR. BRIP1 and RPA32 are reduced in PSPC1 depleted cells



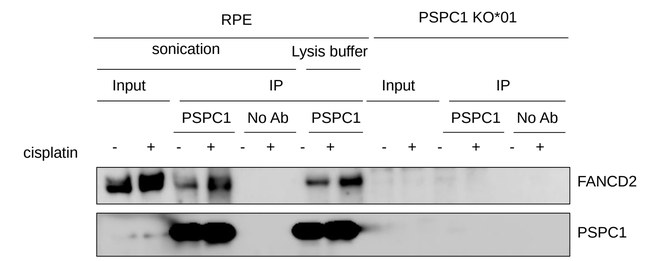
4. RNA levels of Fanconi Anaemia core complex members are reduced in PSPC1 KO cells



5. Loss of DDR proteins in PSPC1 KO cells is not due to proteasomal degradation or transcriptional regulation



6. PSPC1 interacts with FANCD2



Summary

Our results suggest a novel role for PSPC1 in the Fanconi Anaemia pathway. We have shown that PSPC1 is required for phosphorylation of ATR and the protein expression of FANCD2, BRIP1 and RPA32. We illustrate that loss of FANCD2, RPA32 and ATR are not due to transcriptional regulation or proteasomal degradation and PSPC1 can interact with FANCD2. This suggests PSPC1 may have a novel role in the stability of FANCD2 protein.

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