LETTER

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Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair

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Large-scale genomic studies have shown that half of epithelial ovarian cancers (EOCs) have alterations in genes regulating homologous recombination (HR) repair[1]. Loss of HR accounts for the genomic instability of EOCs and for their cellular hyper-dependence on alternative poly-ADP ribose polymerase (PARP)-mediated DNA repair mechanisms[2–5]. Previous studies have implicated the DNA polymerase θ (Polθ also known as POLQ, encoded by *POLQ*)[6] in a pathway required for the repair of DNA double-strand breaks[7–9], referred to as the error-prone microhomology-mediated end-joining (MMEJ) pathway[10–13]. Whether Polθ interacts with canonical DNA repair pathways to prevent genomic instability remains unknown. Here we report an inverse correlation between HR activity and Polθ expression in EOCs. Knockdown of Polθ in HR-proficient cells upregulates HR activity and RAD51 nucleofilament assembly, while knockdown of Polθ in HR-deficient EOCs enhances cell death. Consistent with these results, genetic inactivation of an HR gene (*Fancd2*) and *Polq* in mice results in embryonic lethality. Moreover, Polθ contains RAD51 binding motifs and it blocks RAD51-mediated recombination. Our results reveal a synthetic lethal relationship between the HR pathway and Polθ-mediated repair in EOCs, and identify Polθ as a novel druggable target for cancer therapy.

To examine changes in polymerase activity between tumours and normal tissues, we screened polymerase gene expression profiles in a large number of cancers (Supplementary Table 1). Gene set enrichment analysis (GSEA) revealed specific and recurrent overexpression of Polθ in EOCs (Extended Data Fig. 1a–c). Polθ was upregulated in a grade-dependent manner and its expression positively correlated with numerous mediators of HR (Extended Data Fig. 1d–j). As Polθ has been suggested to play a role in DNA repair[7–10], we investigated a potential role for Polθ in HR repair.

To test the relationship between Polθ expression and HR, we used a cell-based assay in human cells which measures the efficiency of recombination of two GFP alleles (DR-GFP assay)[14]. Knockdown of Polθ with short interfering RNA (siRNA) (Extended Data Fig. 2a) resulted in an increase in HR efficiency, similar to that observed by depleting the anti-recombinases PARI or BLM[15,16]. Depletion of Polθ caused a significant increase in basal and radiation (IR)-induced RAD51 foci (Fig. 1a, b and Extended Data Fig. 2b–d), and depletion of Polθ in 293T cells conferred cellular hypersensitivity to mitomycin C (MMC) and an increase in MMC-induced chromosomal aberrations (Extended Data Fig. 2e, f). These findings suggest that human Polθ inhibits HR and participates in the maintenance of genome stability.

Given that Polθ shares structural homology with coexpressed RAD51-binding ATPases (Extended Data Fig. 1k, l), we hypothesized that Polθ might regulate HR through an interaction with RAD51. RAD51 was detected in Flag-tagged Polθ immunoprecipitates, and purified full-length Flag–Polθ bound recombinant human RAD51 (Fig. 1c, d). Pull-down assays with recombinant GST–RAD51 and *in vitro* translated Polθ truncation mutants defined a region of Polθ binding to RAD51 spanning amino acids 847–894 (Fig. 1e, f and Extended Data Fig. 2g, h). Sequence homology of Polθ with the RAD51 binding domain of *C. elegans* RFS-1 (ref. 17) identified a second binding region (Extended Data Fig. 2i). Peptides arrays narrowed down the RAD51 binding activity of Polθ to three distinct motifs (Fig. 1g and Extended Data Fig. 2j). Substitution arrays confirmed the interaction and highlighted the importance of the 847–894 Polθ region as both necessary and sufficient for RAD51 binding (Extended Data Fig. 3a, b). Together these results indicate that Polθ is a RAD51-interacting protein that regulates HR.

In order to address the role of Polθ in HR regulation, we assessed the ability of wild-type or mutant Polθ to complement the siPolθ-dependent increase in RAD51 foci. Full-length wild-type Polθ fully reduced IR-induced RAD51 foci, unlike Polθ mutated at ATPase catalytic residues (A-dead) or Polθ lacking interaction with RAD51 (ΔRAD51) (Fig. 2a, b). Expression of a Polθ mutant lacking the polymerase domain (ΔPol1) was sufficient to decrease IR-induced RAD51 foci, suggesting that the N-terminal half of Polθ is sufficient to disrupt RAD51 foci (Fig. 2b and Extended Data Fig. 3c, d). We next measured the ability of wild-type or mutant Polθ to complement the siPolθ-dependent increase in HR efficiency. Again, expression of full-length Polθ or ΔPol1 decreased the recombination frequency when compared to cells expressing other Polθ constructs, suggesting that the N-terminal half of Polθ containing the RAD51 binding domain and the ATPase domain is needed to inhibit HR (Fig. 2c and Extended Data Fig. 3e).

A purified recombinant Polθ fragment (ΔPol2) from insect cells exhibited low levels of basal ATPase activity, as previously reported[18] (Fig. 2d, e). Polθ ATPase activity was selectively stimulated by the addition of single-stranded DNA (ssDNA) or fork DNA (Fig. 2e and Extended Data Fig. 4a). Electrophoretic mobility gel shift assays (EMSA) showed specific binding of Polθ to ssDNA (Fig. 2f and Extended Data Fig. 4b). We incubated ΔPol2 with ssDNA and measured RAD51–ssDNA nucleofilament assembly. Interestingly, RAD51–ssDNA assembly was reduced by ΔPol2 wild-type but not by A-dead or ΔRAD51, indicating that Polθ negatively affects RAD51–ssDNA assembly through its RAD51 binding and ATPase activities (Fig. 2g and Extended Data Fig. 4c–f). Furthermore, Polθ decreased the efficiency of D-loop formation, confirming that Polθ is a negative regulator of HR (Fig. 2h and Extended Data Fig. 4g–j).

As Polθ is upregulated in subgroups of cancers associated with HR deficiency (Fig. 3a) and Polθ activity shows specificity for replicative-stress-mediated structures (ssDNA and fork DNA) (Fig. 2e, f), we examined the cellular functions of Polθ under replicative stress. Subcellular fractionation revealed that Polθ is enriched in chromatin in response to ultraviolet (UV) light; and RAD51 binding by Polθ was enhanced by UV

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Supplementary Information is available in the online version of the paper.

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Author Contributions R.C. conceived the study, performed experiments, and wrote the manuscript. J.C.L. and T.Y. purified Polθ fragments from insect cells and performed ATPase and gel shift assays. R.A. performed D-loop formation assays. I.H. and S.J.E. performed the DNA fibres assay. B.P. performed mice work and analysed *in vivo* data. M.I.R.P. and S.J.B. performed the Polθ peptide array and the RAD51–ssDNA filament assembly and release assays. K.W.O. scored RAD51 foci. P.A.K. curated TCGA datasets for Figure 3a and Extended Data Figures 5h and 9e and provided clinical perspectives. A.D.D. conceived the study and wrote the manuscript. All authors approved the final version of the manuscript.

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