DNA polymerase kappa (Pol κ) promotes replication gap suppression by preventing PrimPol mediated repriming in Glioblastoma Multiforme (GBM)

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ABSTRACT

The Y-family translesion polymerase kappa (pol κ) is capable of replicating DNA lesions such as the guanine derivative 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG). Human pol κ is overexpressed in glioblastoma multiforme (GBM), the most aggressive, invasive, and heterogenous form of brain cancer. GBM tumors typically have a high degree of resistance to standard therapeutics, which contributes to poor prognosis. Results from our laboratory demonstrated a role for pol κ in controlling fork speed and suppression of genomic single-stranded DNA (ssDNA) gap formation in GBM cells. Using DNA fiber analysis in multiple cell lines, it was observed that pol κ slows fork elongation rate in GBM-derived cells with no considerable effect in non-GBM cell lines. POLK knockout (POLK-KO) resulted in increased ssDNA gaps only in GBM cells. I have performed experiments that are supportive of the idea that gap formation in pol κ-depleted GBM cells was dependent upon repriming by PrimPol. There was no discernable impact of PrimPol depletion on gap formation in either WT or POLK-KO HAP-1 cells, a commercially available near-haploid cell line derived from chronic myeloid leukemia patient. We found that ssDNA gap formation was reduced in POLK-KO cells in response to treatment with free radical scavenger N-acetyl cysteine (NAC), leading us to hypothesize that pol κ activity is related to the tolerance of oxidative DNA damage in GBM. Experiments are ongoing to determine the exact mechanism by which pol κ action in GBM and how these features might suppress formation of vulnerable ssDNA gaps in GBM.

BACKGROUND

- GBM tumors have a minimal response to standard therapeutics.
- Unrepaired, DNA damage sites like 8-oxo-dG, can contribute to ssDNA gap formation, compromising genome stability.
- TLS pol κ catalyzes bypass of oxidative lesions like 8-oxo-dG, but often pairs 8-oxo-dG incorrectly to adenine.
- Pol κ is upregulated in GBM cells, but the impact on fork dynamics and the mechanistic features leading selection for pol κ are not fully understood.

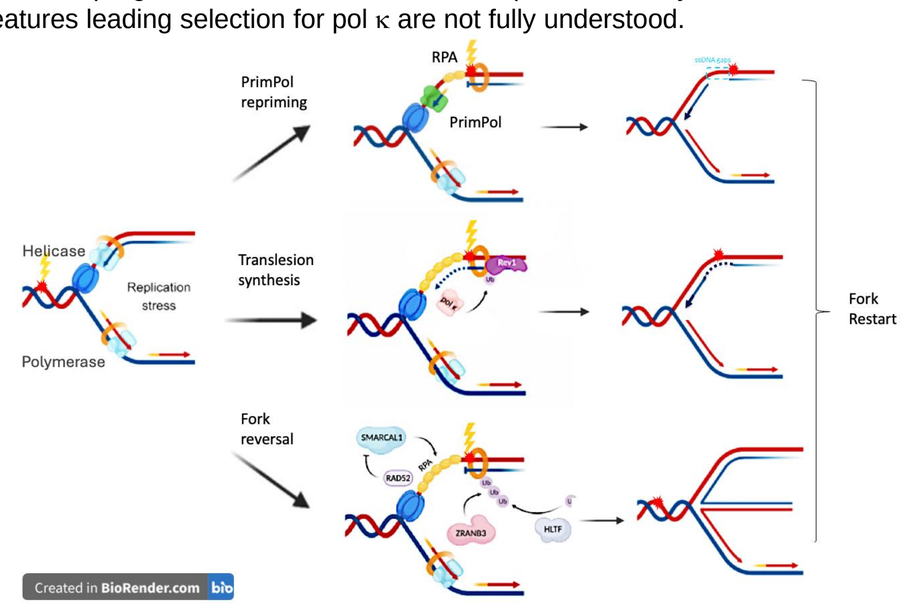

Fig. 1. Modes of DNA damage tolerance. Upon stalling of the replication fork, cells activate DDT pathways to repair and restart the fork to maintain genome integrity. DNA lesions can also be bypassed by translesion synthesis primase and DNA polymerase (PrimPol) that mediates repriming of DNA synthesis a head of DNA polymerase. Alternatively, TLS can be performed by specialized DNA polymerases, such as those in the Y-family, capable of performing DNA synthesis across damaged templates. Fork reversal can occur at the stalled fork to regulate fork speed.

HYPOTHESIS

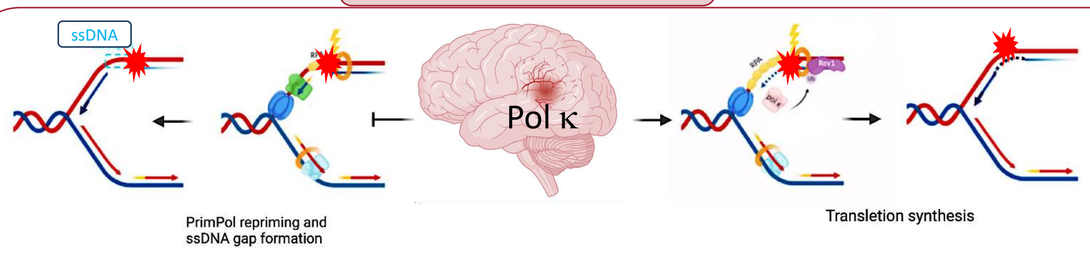

Fig. 2. Proposed models for hypothesis. Pol κ prevents accumulation of ssDNA gaps by performing TLS to oppose repriming by PrimPol.

METHODS

DNA fiber spreading displays of replication fork dynamics

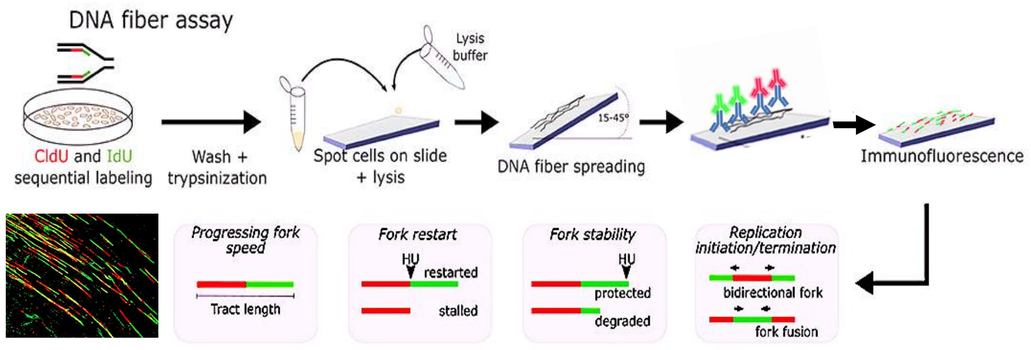

Fig. 3. Visualization of replication fork dynamics by DNA fiper assay. Overview of experimental scheme of DNA fiber spreading step by step.

RESULTS

pol κ plays an important role in controlling fork speed and ssDNA gaps accumulation in GBM

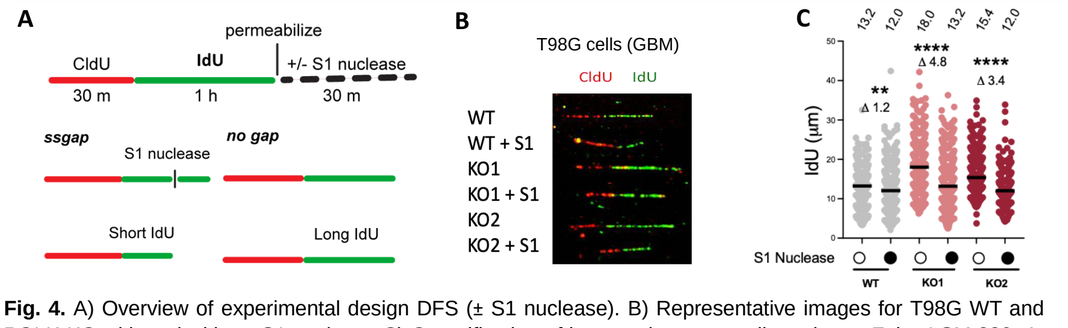

Fig. 4. A) Overview of experimental design DFS (± S1 nuclease). B) Representative images for T98G WT and POLK-KO with and without S1 nuclease C) Quantification of images that were collected on a Zeiss LSM 880. At least 150 fibers were scored per condition from 3 biological replicates using ImageJ. The mean track length is noted for each condition along with the S1-dependent change (Δ).

FUNDING

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RESULTS (CONT.)

The formation of ssDNA gaps in pol κ-depleted GBM cells is dependent upon repriming by PrimPol

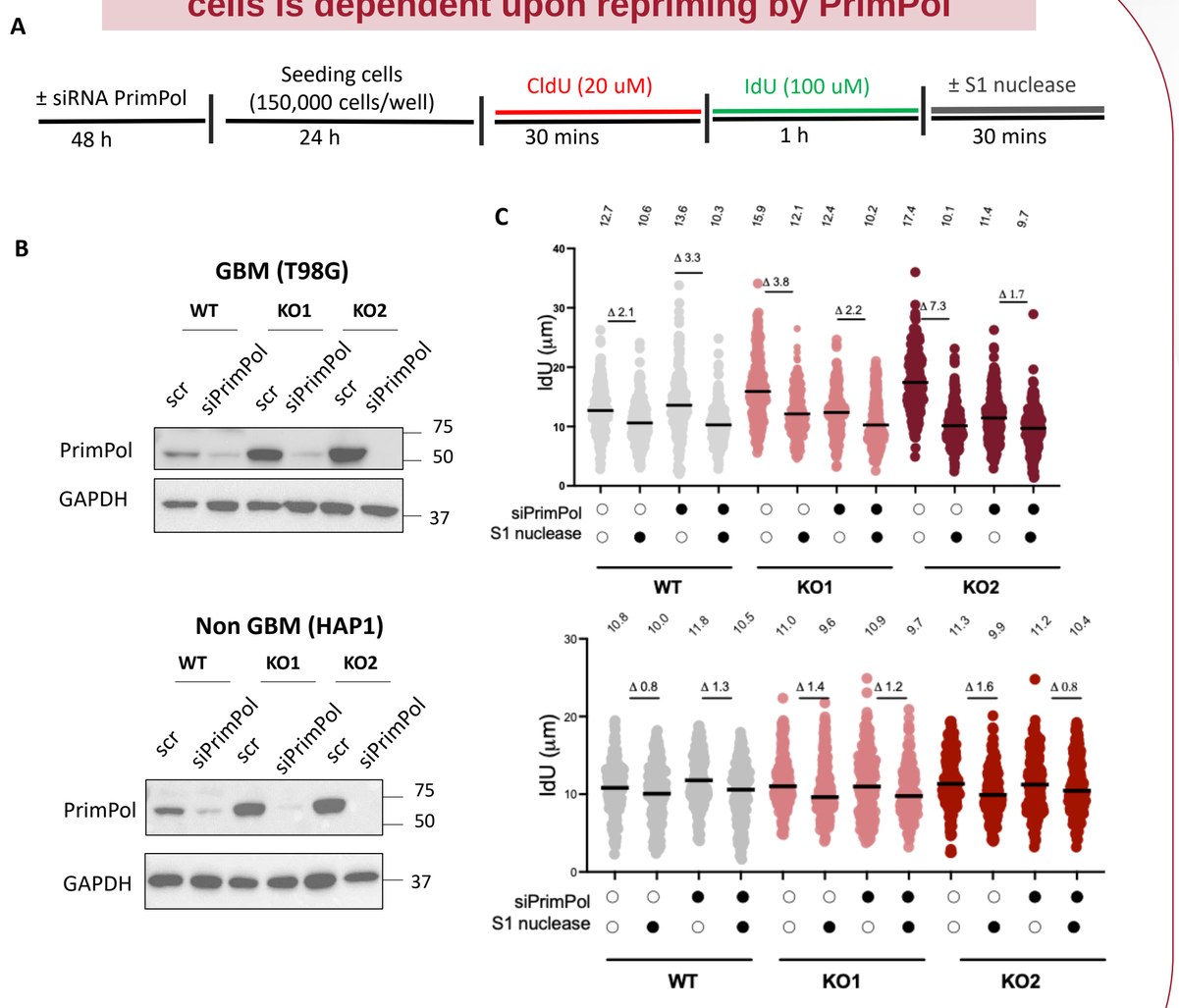


Fig. 5. A) Overview of experimental design DFS (± S1 nuclease) for detection ssDNA gap formation with and without PrimPol depletion B) T98G and HAP1 WT and POLK-KO were transfected with nontargeting (scramble) or PrimPol siRNAs. C) Quantification of images that were collected on a Zeiss LSM 880. At least 100 fibers were scored per condition from 2 biological replicate, 3 technical replicates using ImageJ.

Pol κ plays a role in the tolerance of redox imbalance in GBM.

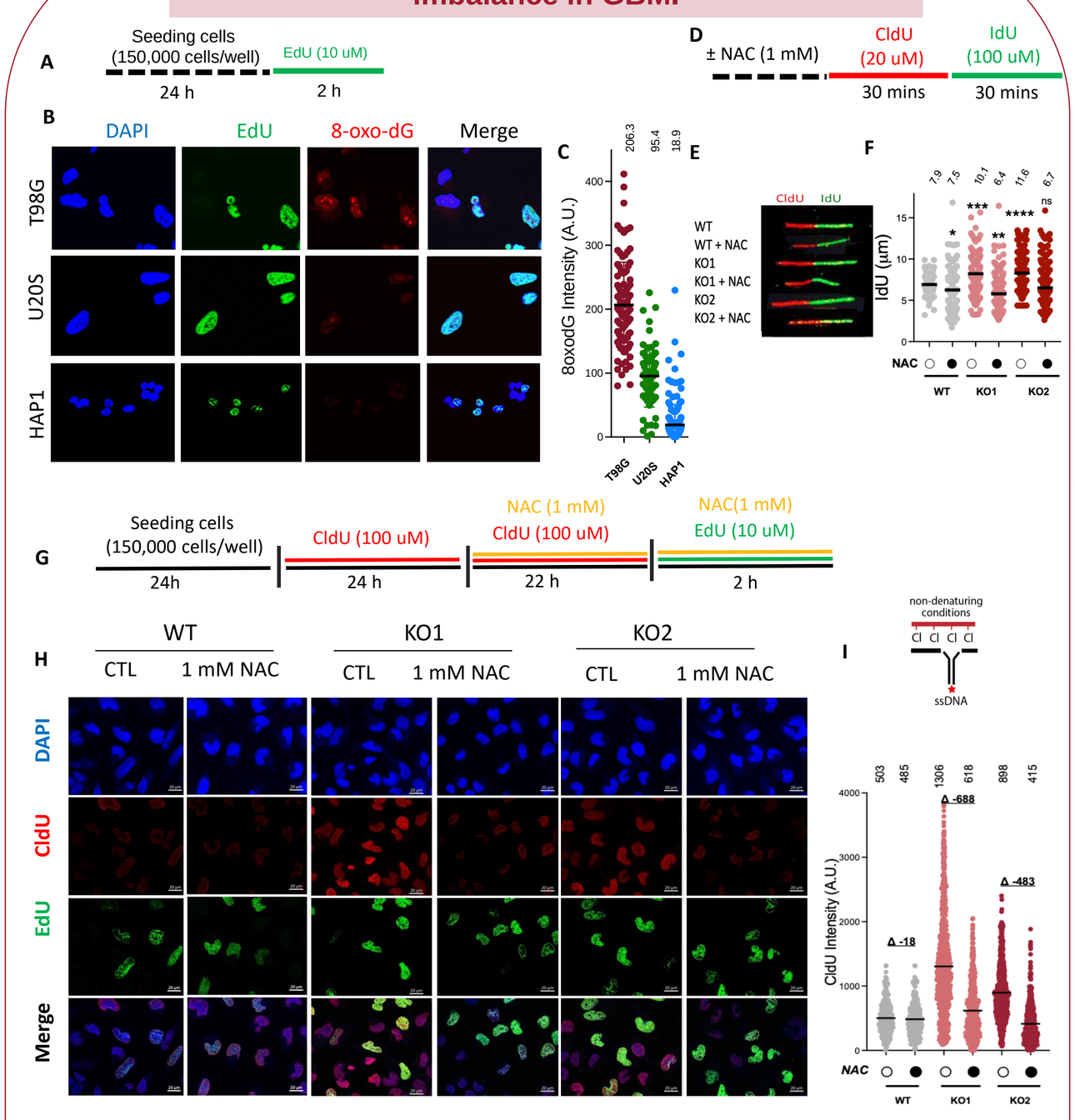
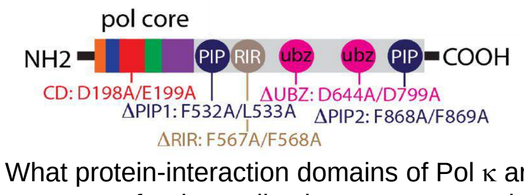


Fig. 6. A) Overview of experimental design for detection of 8oxodG level in T98G, U2OS, and HAP1 cells. B) Representative images for detection of 8-oxo-dG intensity in multiple cells. C) Quantification of images that were collected on Olympus. At least 65 cells were counted per condition. D) Overview of experimental design DFS for detection fork elongation rate in response to NAC. E) Representative images measuring fork speed by measuring IdU track length in T98G WT and POLK-KO cells in response to treatment ± 1 mM NAC. F) Quantification of images that were collected on a Zeiss LSM 880. At least 150 fibers were scored per condition from 2 biological replicates, 3 technical replicates using ImageJ. G) Overview of experimental design for non- denaturing dual pulse CldU- EdU IF experiment for detection ssDNA gap formation in response to NAC. H) Representative images measuring of ssDNA gap formation by monitoring CldU signal in T98G WT and POLK-KO cells in response to treatment ±1 mM NAC. I) Quantification of images that were collected on a Zeiss LSM 880. At least 1000 cells were scored per condition from one biological replicate and quantified using ImageJ and CellProfilerTM. Fluorescence intensity was plotted in PrismGraph Pad.

CONCLUSION

- Pol κ controls fork speed and replication gap suppression in GBM cells.
- The accumulation of ssDNA gaps in pol κ -deficient cells is dependent upon repriming by PrimPol in GBM cells with no considerable effect on non-GBM cells.
- Pol κ could play a role in the tolerance of oxidative damage that accompanies redox imbalance in GBM.

FUTURE DIRECTIONS



What protein-interaction domains of Pol κ are necessary for the replication gap suppression phenotype?