results

DNA polymerase η (hPol η) is a key protein in translesion DNA synthesis (TLS) in human cells. Its primary function is the error-free replication through UV-induced TT cytosine dimers which present a barrier to DNA synthesis by other eukaryotic replicative polymerases. hPol η defects underlie the genetic disease seckel syndrome variant (XPV) characterized by higher susceptibility to UV-light induced skin cancers due to erroneous replication of the UV adducts. However, hPol η is also a very low-fidelity enzyme when copying undamaged DNA or DNA with other adducts and is actively recruited during the somatic hypermutation of the immunoglobulin genes.

We demonstrate that hPol η restores partially the mutability and completely the survival of a UV non-mutable umuDC-Δleth mu1 strain of Escherichia coli after UVB irradiation. The catalytic core lacking the C-terminal part of hPol η was even more biologically active than the full size protein and its activity was further enhanced by attaching the prokaryotic S-subunit binding motif to it. The mutagenic and survival effects were enhanced upon the induction of HPol η expression and its catalytically inactive variant was unable to promote any mutagenesis. This suggests that HPol η directly participates in the replication of damaged DNA in the prokaryotic bacteria.

To demonstrate that our system can be useful in studying different variants of hPol η in vivo we have constructed 4 amino acid substitution mutants with altered geometry of the catalytic site analyzed previously biochemically and confirmed their altered abilities to promote mutagenesis and survival after UVB irradiation. This study paves a way to generate a variety of useful derivatives of hPol η in prokaryotic systems.

Conclusions

Human DNA polymerase η (hPol η) is the most well studied Y-family DNA polymerase and its activity is mechanistically involved in the initiation of TLS associated with the BRCAl mutation.

We have successfully expressed hPol η in the prokaryotic model bacterium Escherichia coli as confirmed by Western blotting and response to IPTG induction.

hPol η remained functional in E. coli and participated in the translesion DNA synthesis across DNA lesions induced by UVB irradiation.

The activity of hPol η in E. coli was remarkably increased by removing its C-terminal part carrying motifs specific to eukaryotes and completely abolished by substituting 2 essential residues within its catalytic site.

Chimeric hPol η proteins carrying the translesion DNA targeting motifs showed increased ability to promote UVB-mutagenesis.

A single amino acid substitution within the motif I of finger domain (SSGQ) significantly enhanced hPol η abilities to increase survival and mutagenesis after UVB irradiation in vivo.

The hPol η'sclile site mutant (F18A), although biologically active in vitro, completely lost its activity in vivo.

Substitution of the amino acid residue causing the bso-2DTP mimicking corosporine insensitive template sl in vitro (R61) produced mutants of hPol η which lost the ability to promote mutagenesis at low expression level in vivo and suppressed the survival after UVB irradiation.

Our new system allows for the generation and analysis of the effects of various hPol η mutants on the enzyme's functionality during TLS in E.coli as well as for testing the intrinsic ability of hPol η to modulate TLS across other types of DNA adducts in vitro.

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