Human DNA polymerase β polymorphism, Arg137Gln, impairs its polymerase activity and interaction with PCNA and the cellular base excision repair capacity

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ABSTRACT

DNA polymerase β (Pol β) is a key enzyme in DNA base excision repair, and an important factor for maintaining genome integrity and stability. More than 30% of human tumors characterized to date express DNA Pol β variants, many of which result from a single nucleotide residue substitution. However, in most cases, their precise functional deficiency and relationship to cancer susceptibility are still unknown. In the current work, we show that a polymorphism encoding an arginine to glutamine substitution, R137Q, has lower polymerase activity. The substitution also affects the interaction between Pol β and proliferating cell nuclear antigen (PCNA). These defects impair the DNA repair capacity of Pol β in reconstitution assays, as well as in cellular extracts. Expression of wild-type Pol β in *pol β⁻/⁻* mouse embryonic fibroblast (MEF) cells restored cellular resistance to DNA damaging reagents such as methyl methanesulfonate (MMS) and *N*-methyl-*N*-nitrosourea (MNU), while expression of R137Q in *pol β⁻/⁻* MEF cells failed to do so. These data indicate that polymorphisms in base excision repair genes may contribute to the onset and development of cancers.

INTRODUCTION

Genomic DNA is constantly exposed to endogenous and exogenous insults, which react with base and sugar groups causing DNA damage which, if not repaired, may result in genetic mutations, and subsequently contribute to genome instabilities and cancer initiation (1,2). Removal of DNA damage and maintenance of genomic integrity depend on robust cellular DNA repair systems (2). Base excision repair (BER) is one of the major repair pathways in eukaryotic cells, processing DNA base damage caused by endogenous and exogenous agents (3,4). It is estimated that BER is responsible for repairing about 10^4 damaged/modified bases per cell per day (5–7). BER is initiated with the excision of the damaged base by a specific DNA glycosylase, followed by incision of the DNA backbone by AP endonuclease 1 (APE1) to produce a nicked abasic intermediate (8). This intermediate structure can be processed through either the short-patch BER (SP-BER) or the long-patch BER (LP-BER) pathway (9,10). In the former, DNA polymerase β (Pol β) adds only one nucleotide to the 3′-end of the nicked AP site, and then the dRP lyase activity of Pol β catalyzes β-elimination of the 5′-sugar phosphate residue, resulting in a ligatable nick that can then be sealed by XRCC1/Ligase IIIa (11,12). In the latter, Pol β or the alternative Pol δ performs strand displacement synthesis, generating a short DNA flap of 2–10 nt, which is removed by flap endonuclease 1 (FEN1) (13–17). DNA ligase I then seals the nick (9).

Of the tens of BER proteins identified, Pol β has been demonstrated to be a key player in both SP- and LP-BER (18,19). Pol β, a 39 kDa protein, contains two domains; a dRP lyase domain (8 kDa) and a polymerase domain (31 kDa). These two domains correspond to the dRP lyase and polymerase activities, which are responsible for the removal of the sugar phosphate group and the incorporation of new deoxyribonucleotides, respectively (20). In addition, Pol β also interacts with many other proteins including XRCC1 (21–25), proliferating cell nuclear antigen (PCNA) (26), and FEN1 (27,28). These interactions likely play important roles in recruiting downstream factors to the DNA repair site, reciprocally stimulating enzyme activities and coordinating the highly ordered chemical reactions of BER. Pol β deficiency has been demonstrated to impair BER efficiency and to cause cells to be hypersensitive to alkylating or oxidative agents (15,29). Knockout of Pol β in mice abolishes

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BER and causes the mutant cell to be hypersensitive to DNA damaging reagents, including methyl methanesulfonate (MMS) and *N*-methyl-*N*-nitrosourea (MNU), leading to early embryonic lethality. Pol β mutations that affect its dRP lyase, polymerase activity or its interaction with other proteins, have been shown to result in defective BER *in vitro* (30–33).

Single nucleotide polymorphisms occur widely in DNA repair genes (34). While previous research suggests polymorphisms may result in biochemical alternations, BER deficiency and predisposition to cancers (31,34–37), it is still of significant interest to determine whether and how a polymorphism increases the susceptibility to development of cancers. For DNA Pol β, three non-synonymous single nucleotide substitutions were identified as polymorphisms in humans. These are Q8R, R137Q and P242R. However, it is unknown whether the polymorphism affects Pol β's function and contributes to cancer initiation and development. Among the three DNA Pol β polymorphisms, R137Q is particularly interesting because the residue at position 137 locates in the helix 7 of Pol β protein (38) and forms salt bridges with other adjacent amino acid residues. The amino acid substitution of Arg by Gln results in a net positive charge loss and might lead to significant alterations in biochemical and physiological properties of the enzyme. Moreover, a recent report showed that R137 is also a methylation site of Pol β (39). In the current study, we expressed and purified the R137Q Pol β variant and found that R137Q significantly reduced polymerase activity. It also showed impaired interaction with PCNA. As a result, the R137Q substitution reduced BER efficiency when assayed in a reconstitution assay or with cellular extracts. Furthermore, mouse cells expressing the R137Q variant accumulated DNA damage lesions in the genome and were sensitive to DNA damaging agents. These results suggest that the R137Q polymorphism affected Pol β biochemical activity resulting in defective BER, which might subsequently contribute to genome instability and cancer development.

EXPERIMENTAL PROCEDURES

Preparation of recombinant wild-type and variant Pol β

The wild-type (WT) human *pol β* cDNA was cloned from a human cDNA library. R137Q substitution was obtained by site-directed mutagenesis using the primers shown in Table 1. WT and R137Q genes were inserted into the pET28b vector and expressed in *Escherichia coli* strain BL21. Expressed proteins were purified as previously described (40).

In vitro polymerase activity assay

The polymerase activity assay utilized 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 20 mM NaCl, 10% glycerol, 0.1 μM biotin-labeled DNA substrate Pol-GAP (see Table 1 and Figure 1A for detail), 50 μM each dATP, dGTP and dTTP (Sigma), 8 μM 2 μCi [α-^{32}P]-dCTP and 0–10 ng WT or R137Q Pol β. Reactions were carried out at 37°C for 30 min. A portion of reaction product was taken out and incubated with avidin–Sepharose

4B beads, washed and quantified on liquid scintillation analyzer. The left portion of reaction product was then stopped by addition of equal volumes of gel loading buffer (90% formamide dye, 3 M EDTA, 0.02% bromophenol blue and 0.02% xylene cyanol), heated (5 min, 95°C), separated by 15% Polyacrylamide gel electrophoresis (PAGE) containing 8 M urea and visualized by autoradiography.

DNA-binding assay

Gel shift and ELISA-based isothermal absorption assays were used to compare the DNA-binding affinity between WT and R137Q Pol β. For gel shift assays, various concentrations of Pol β protein (0.1–1000 nM) were incubated (15 min, room temperature) with 0.1 nM radio-labeled Pol-GAP substrate in a buffer containing 50 mM Tris–HCl (pH 8.0), 100 mM NaCl, 10 mM $MgCl_2$, 10% glycerol and 0.1% NP-40. Samples were run on a 5% native polyacrylamide gel and visualized by autoradiography. For ELISA-based affinity assays, biotin-labeled Pol-GAP DNA substrate (1 pmol) was immobilized on a streptavidin-coated 96-well Enzyme-Linked ImmunoSorbent Assay (ELISA) plate and washed three times with binding buffer, followed by the addition of 0–1 μg WT or R137Q Pol β and incubated (overnight, 4°C). Bound Pol β was detected by mouse anti-Pol β antibody (Genetex, GTX23181) and goat anti-mouse IgG/HRP (Genetex, GTX85313). Color was developed by adding tetramethyl benzidine (TMB) and stopped by addition of 1 N HCl. The OD_{450} was read on a microplate reader.

dRP lyase assay

The dRP lyase assay using purified Pol β protein was performed as described previously (41). To prepare the dRP lyase substrate, the DNA substrate Pol β-U was labeled with (γ-^{32}P)-ATP at the 5′-end on the U-containing oligonucleotide, followed by treatment with uracil-DNA glycosylase (UDG) and APE1. The incised AP-site-containing DNA was then incubated (20 min, 37°C) with Pol β (0–10 ng) in a buffer containing 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 20 mM KCl and 2 mM DTT, stabilized by addition of 2 M sodium borohydride to a final concentration of 340 mM, followed by incubation on ice (30 min). Formamide-containing gel loading buffer was then added and reaction products were resolved on 15% polyacrylamide gels containing 8 M urea and visualized by autoradiography.

Reconstituted base excision repair assay

The BER assay was performed as described previously (22,42,43). Complete repair reactions were carried out in 20 μl of reaction buffer [40 mM HEPES–KOH (pH 7.8), 70 mM KCl, 7 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM EDTA, 2 mM ATP, 50 μM each of dATP, dTTP and dGTP, and 8 μM 2 μCi (α-^{32}P)-dCTP]. For short-patch reconstitution with purified proteins, UDG (8 ng), APE1 (2 ng), Ligase IIIα (20 ng) and various amounts of Pol β (0–5 ng), were mixed and incubated with the SP-BER substrate Pol β-U (Table 1). For LP BER, Pol β-F substrate (Table 1) was incubated with the mixture of APE1

Table 1. Oligonuleotide sequences and their applications in this study

Name	Oligonuleotide sequence	Application
Pol β R137Q F	5′-gat aaat t gaaccat cagcaaat t gggct gaaat t t t ggg-3′	Mutagenesis
Pol β R137Q R	5′-cccaaaat at t t cagcccaat t t gct gat gat ggt t caa t t ta tc-3′	
Pol-GAP	5′-ct t acacgt t gact accgt t t t t gaggcaagagt ggat cc-3′[a]	Polymerase activity and DNA binding assay
	3′-gaat gt gcaact gat ggcagaaac t ccgt t c t cacc t agg-5′	
Pol β-U	5′-c t tacacg t tgac tacc t tUt t tgaggcaagag t gga t cc-3′[b]	SP-BER and dRp lyase assay
	3′-gaat gt gcaact gat ggcagaaac t ccgt t c t cacc t agg-5′	
Pol β-F	5′-c t tacacg t tgac tacc t t Ft t tgaggcaagag t gga t cc-3′[c]	LP-BER assay
	3′-gaat gt gcaact gat ggcagaaac t ccgt t c t cacc t agg-5′	

[a]P: phosphate group.
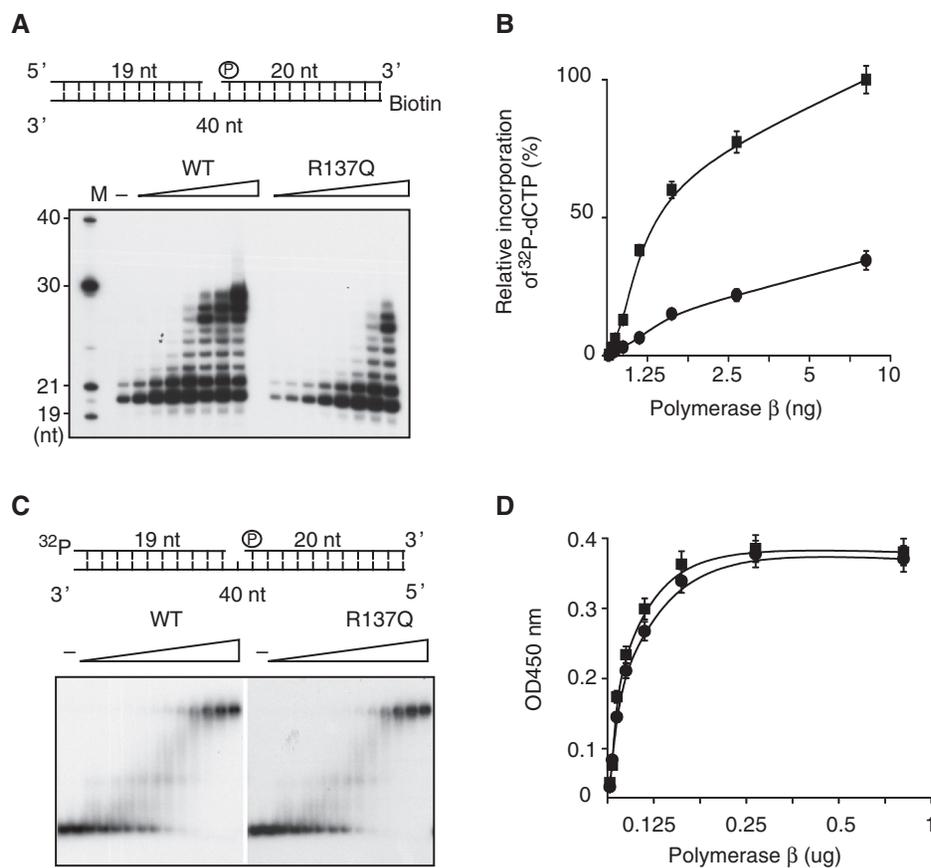[b]U: uracil.
[c]F: Tetrahydrofuran (THF).

Figure 1. The Pol β polymorphism R137Q is defective in polymerase activity. (**A**) Top panel, schematic of the biotin-labeled 1-nt gapped DNA substrate (Pol-GAP); bottom panel, polymerase activity assay in which Pol-GAP was incubated with 50 µM each of dATP, dGTP, dTTP and 8 µM dCTP-^{32}P and varying amounts of purified Pol β protein (WT and R137Q). WT and R137Q DNA polymerization products were separated by denaturing gel electrophoresis and visualized with a phosphorimager. (**B**) DNA polymerization products were pulled down by Sepharose–avidin beads. After washing, the amount of radio-nucleotide incorporated into the products was determined by liquid scintillation counting. (**C**) Gel shift assay of DNA-binding affinity of R137Q and WT Pol β. In this assay, Pol-GAP was labeled by ^{32}P as shown in figure. (**D**) ELISA-based isotherm adsorption assay of DNA-binding affinity of R137Q and WT Pol β. The DNA substrate was the same as that used in (A). WT, filled squares; R137, filled circles.

(2 ng), Pol β (0–5 ng), FEN1 (2 ng), and Ligase I (20 ng). Note that in the LP-BER substrate, radio-labeled dCTP can only be incorporated in the second position next to the damaged base. For cell extract reconstitution, SP- or LP-BER DNA substrate was incubated with the total cell extracts (0–5 µg). Reactions (30 min, 37°C) were then stopped by adding an equal volume of the gel loading buffer and visualized by autoradiography.

Cell lines and culture

WT and *pol β* null (*pol β*$^{-/-}$) mouse embryonic fibroblast (MEF) cells were described previously (15). Cells were

grown in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% FBS and 100 units each of penicillin, streptomycin and hygromycin. The *pol β*$^{-/-}$/ WT and *pol β*$^{-/-}$/R137Q stable cell lines were generated by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Selection was carried out using 100 µg/ml of G418.

Preparation of cell extracts

Cell extracts were made according to Biade *et al.* (43). Briefly, cells were cultured in 10 cm^2 dishes, incubated overnight to reach a mid-exponential growth phase, washed three times with ice-cold PBS and resuspended at 10^6 cells/20 µl in buffer I [10 mM Tris–HCl (pH 7.8) and 200 mM KCl]. After the addition of an equal volume of Buffer II [10 mM Tris–HCl (pH 7.8), 200 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% NP-40, 2 mM dithiothreitol, 0.5 mM PMSF and protease inhibitor cocktail (Roche)], the cell suspension was rocked (1 h, 4°C), centrifuged (10 min, 16 000g) and the supernatant recovered and stored (−80°C). Protein concentrations were determined by Bradford protein assay.

Pull-down assay

To determine the interaction between R137Q and proteins in the BER complex, His-tagged Pol β protein (20 µg) was mixed with cell extract prepared from the *pol β*$^{-/-}$ MEF cell line (50 µg) in 150 µl binding buffer [50 mM Tris–HCl (pH 7.6), 75 mM KCl, 1 mM DTT, 10 mM imidazole], incubated (60 min, on ice) and 15 µl of pre-equilibrated nickel beads were added to the mixture which was once again incubated (1 h, on ice, with constant shaking). Beads were then washed five times with the binding buffer, suspended in SDS–PAGE sample buffer (50 µl) (31), boiled (5 min, 95°C) and the supernatant subjected to SDS–PAGE followed by western blotting with antibodies against APE1 (Genetex, GTX70132), FEN1 (Genetex, GTX70186), PCNA (Genetex, GTX70286), Ligase IIIα (Genetex, GTX70144), Ligase I (Genetex, GTX70142) and XRCC1 (Genetex, GTX70262). To confirm the interaction between Pol β and PCNA, purified PCNA was immobilized on Sepharose 4B, as previously described (40); PCNA coated beads were then mixed with purified WT or R137Q Pol β. After incubation (60 min, on ice) and washing with binding buffer, Sepharose 4B beads were boiled in SDS–PAGE sample buffer and the supernatant analyzed by western blotting with anti-Pol β and PCNA antibodies.

DNA damage assay

DNA damage assay was carried out as described (44). Briefly, monolayer cells were treated (1 h) with MMS at different concentrations, collected and genomic DNA was extracted by using DNAzol reagent (Invitrogen). Extracted DNA was then coated onto ELISA plates and detected by AP-site specific reagent, Aldehyde-Reactive Probe (ARP) (Invitrogen), followed by detection with HRP-conjugated streptavidin and TMB. After stopping by 1N HCl, OD$_{450}$ was measured on a microplate reader.

MMS and MNU sensitivity assay

Sensitivity to MMS and MNU was determined by HeLa cell growth inhibition assay. HeLa cells were seeded (1500/well), incubated (overnight, 37°C). Treated (1 h, 37°C) with multiple dilutions of MMS, washed in a fresh medium (DMEM containing 10% fetal bovine serum) and incubated (72 h) under normal growth conditions (37°C, 5% CO$_2$). The number of viable cells was determined by the CellTiter 96 AQueous one-solution cell proliferation assay (Promega). At least four replicas for each clone were averaged. Data are expressed as the percentage of growth relative to untreated controls.

Cell apoptosis analysis

The cell cycle distribution and apoptosis of stably complemented cell lines were analyzed by Fluorescence Activated Cell Sorting (FACS) analysis, as previously described (45). Harvested cells (10 cm^2 dish) were fixed with pre-chilled 70% ethanol, stained with propidium iodide (PI; Sigma) and analyzed by flow cytometry at the City of Hope Flow Cytometry Core Facility.

RESULTS

The R137Q variant is defective in polymerase activity

The Pol β polymorphism (rs12678588) encoding an Arg137 to Gln substitution was initially identified by the Human Genome Project and was confirmed by Mohrenweiser *et al.* (46). This polymorphism has been identified in Caucasians, Africans and Asians with rare occurrence frequency (National Center for Biotechnology Information database and reference 38). However, the extent of the impact of this polymorphism on disease development, especially carcinogenesis, has not been fully elucidated. To determine the effects of the R137Q Pol β variant on biochemical activities and/or biological functions, we first expressed and purified WT and R137Q human Pol β proteins, and then assayed the primer extension, dRP lyase and substrate-binding activity *in vitro*, using synthetic DNA substrates (Table 1). We found that the R137Q variant had ∼30% primer extension activity compared to the WT enzyme (Figure 1A and B). However, no differences were observed in the R137Q variant for the DNA-binding activity or dRP lyase activity (Figures 1C, D and 2). These results were consistent with the fact that R137Q occurred at the 31-kDa domain, the polymerase catalytic domain, while the 8-kDa domain was responsible for dRP lyase and DNA-binding activity.

The R137Q variant has an impaired interaction with PCNA

We then investigated the effects of R137Q substitution on protein–protein interactions. Extracts of *pol β*$^{-/-}$ MEF were incubated with purified WT or R137Q proteins (His-tagged) prior to immunoprecipitation of proteins involved in the BER pathway and detection by corresponding antibodies (Figure 3). Six proteins (APE1, FEN1, PCNA, XRCC1, Ligase I and Ligase IIIα) that interact with Pol β and are involved in either the
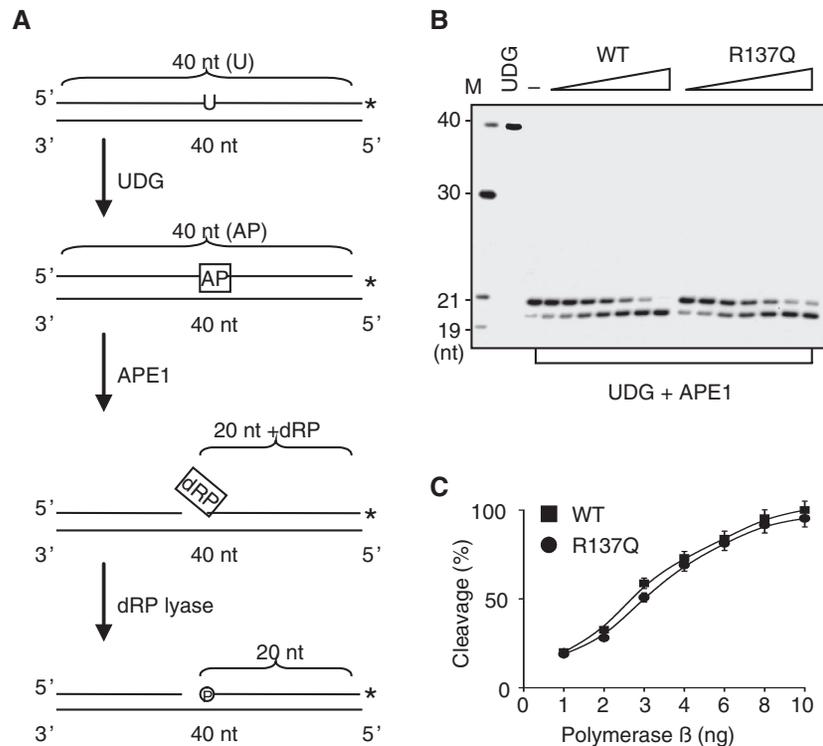
Figure 2. dRP lyase activity assay of Pol β. (**A**) Schematic representation of the reactions generating a 5′-dRP-group-containing DNA substrate. Treatment of Pol β-U (with ^{32}P labeled at 3′end) with UDG generates an AP-containing DNA, which then was incised at the nucleotide 5′ to the AP site by APE1, resulting the 5′-dRP-containing substrate (20 nt + dRP). Removal of the 5′-dRP residue by dRP lyase results in a 3′-^{32}P labeled 20 nt DNA fragment. Asterisk represents ^{32}P labeling. (**B**) 5′-dRP lyase activity assay. (**C**) Quantification of the 5′-dRP lyase activities shown in (**B**).

SP- or LP-BER pathways were investigated. The amount of APE1, FEN1, XRCC1, Ligase 1 or Ligase IIIα that co-pulled down with R137Q was similar to that with the WT enzyme. However, there was a dramatic decrease in levels of R137Q-bound PCNA compared with WT Pol β-bound PCNA (Figure 3A, panel 2). To rule out the possibility that the His tag itself is responsible for the observed binding, we included His-tagged glutathione *S*-transferase (GST) as a control. In this case, none of the tested proteins binds to His-tag or GST. To further confirm this observation, we immobilized purified PCNA on Sepharose 4B beads and incubated them with purified WT or R137Q Pol β. Western blot of Sepharose bead-bound proteins showed that WT Pol β but not R137Q was efficiently pulled down by PCNA (Figure 3B), suggesting that the Arg137 to Gln substitution impaired the interaction between Pol β and PCNA. To quantitatively evaluate the impact of the R137Q substitution on the Pol β/PCNA interaction, we determined the relative amount of WT Pol β or R137Q-bound PCNA by ELISA. Our data suggested that levels of WT Pol β-bound PCNA were 1.5- to 4-fold higher than those of R137Q-bound PCNA (Figure 3C).

The R137Q variant has lower BER efficiency

The impaired biochemical functions of the R137Q variant suggested that R137Q likely affected BER function. To test this hypothesis, SP- and LP-BER were assayed using purified proteins. Uracil-containing substrate (Pol β-U) and tetrahydrofuran-containing substrate (Pol β-F) were used for SP- and LP-BER substrates, respectively (Table 1). Cleavage of the uracil or Tetrahydrofuran (THF) lesion by the concerted action of UDG and APE1 resulted in a nicked DNA duplex, and incorporation of ^{32}P-dCTP and other deoxynucleotides produced 20–30 nt non-ligated intermediates visible to the phosphorimager (Figure 4A and B). Further processing of this intermediate structure generated a fully repaired product of 40 nt. We observed that the uracil or THF lesions were efficiently repaired in the presence of WT Pol β but not the R137Q variant (Figure 4A and B). The reduced repair efficiency by R137Q was likely due to its low-polymerase activity. Consistent with the polymerase activity assays (Figure 1A), only a small amount of ^{32}P-dCTP was incorporated into the DNA substrate by R137Q in reconstituted SP- and LP-BER reactions (Figure 4A and B). To validate that the R137Q variant lead to low-BER efficiency, we expressed human WT and R137Q Pol β in mouse MEF of *pol β* knockout genetic background. Cells expressing similar level of Pol β were selected (Figure 5A). Nuclear extracts (NEs) from these cell lines were prepared and their BER efficiency assayed. We found that WT NE efficiently repaired the uracil or THF lesion, resulting in a 40 nt band, whereas the repair efficiency by R137Q NE was only ∼10 and 20% for SP- and LP-BER, respectively (Figure 5B and C).
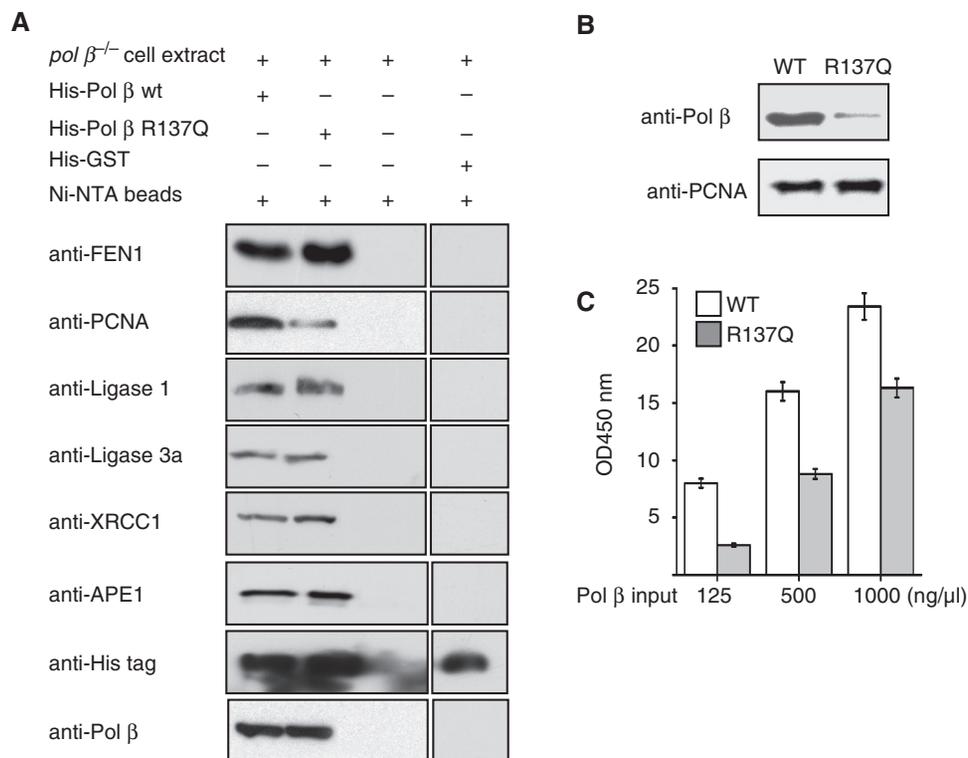
Figure 3. The R137Q Pol β variant has an impaired interaction with PCNA. (**A**) Ni-NTA pull-down was performed with the cell extracts from *pol β* null MEFs and purified Pol β or R137Q variant proteins (His-tagged). The interaction was detected by western blotting using antibodies against FEN1, APE1, PCNA, Ligase 1, Ligase IIIα and XRCC1. A negative control with His-tagged GST was added to rule out the possibility that desirable proteins interact with His tag directly. (**B**) Pull-down assay using purified proteins. Purified PCNA was immobilized on Sepharose 4B beads, incubated with purified Pol β (WT or R137Q), washed and beads were then boiled in SDS–PAGE sample buffer. Proteins were separated by SDS–PAGE and transferred onto PVDF film, followed by detection with anti-Pol β and anti-PCNA antibodies. (**C**) Affinity comparison of PCNA with WT and R137Q Pol β by ELISA. PCNA was coated onto ELISA plates, incubated with different concentrations of purified WT or R137Q Pol β, washed and bound protein was detected by mouse anti-Pol β antibody and goat anti-mouse IgG/HRP. Color was developed by adding TMB. The color reaction was stopped by adding 1 N HCl. Optical density was read on microplate reader.

Expression of the R137Q variant in *pol β* knockout cells failed to restore DNA damage resistance capacity

Our observation that R137Q Pol β caused defective BER led us to propose that cells expressing the R137Q variant would be sensitive to DNA base damaging agents. To test this hypothesis, MEF cells of WT, *pol β* knockout (*pol β$^{-/-}$*) and *pol β$^{-/-}$* expressing human WT or R137Q Pol β (designated as *pol β$^{-/-}$*/WT or *pol$^{-/-}$*/R137Q) were treated with MMS. Consistent with a previous report (15), we found that deletion of *pol β* caused cells to accumulate MMS-induced DNA damage at MMS concentrations ≥1 mM (Figure 6A) and to become sensitive to the MMS and MNU (Figure 6B–D). While human WT Pol β fully complemented the function of its mouse homolog, R137Q only partially complemented it. FACS data showed that apoptotic cells of *pol β* knock out or *R137Q* genetic background were considerably higher than the WT background. Additionally, there was a 5-fold increase in MMS-induced apoptotic R137Q cells compared with WT (3.1% versus 0.6%) (Figure 6B). MMS consistently had a stronger inhibitory effect on *pol β$^{-/-}$*-R137Q cell growth relative to WT or *pol β$^{-/-}$*/WT cells. Following MMS or MNU treatment, the ratio of MMS or MNU treated/untreated *pol β$^{-/-}$*-R137Q cells

was <10%, whereas that for WT or *pol β$^{-/-}$*-WT cells was >90% (Figure 6C and D).

DISCUSSION

Pol β plays a key role in the DNA BER pathway and is critical for normal cell function. Approximately 30% of human cancers contain abnormal Pol β (47). Cancer-associated somatic mutations of *pol β* usually induce genomic instability and lead to cellular transformation when expressed in mouse fibroblasts (31,48,49). Unlike somatic mutations found in cancer patients, polymorphisms exist in normal populations and are hypothesized to be liable for the onset of cancer. Given the importance of Pol β in maintaining DNA integrity, we were interested in providing mechanistic insights associated with cancer development of a newly identified polymorphism of Pol β, R137Q. Here, we show that the R137Q variant decreases Pol β polymerase activity and impairs its interaction with PCNA and the overall capacity to repair DNA base damage *in vitro* and *in vivo*. Our study suggests that an individual with R137Q polymorphism may be sensitive to endogenous and exogenous DNA damaging agents, and consequently be susceptible to cancer development.
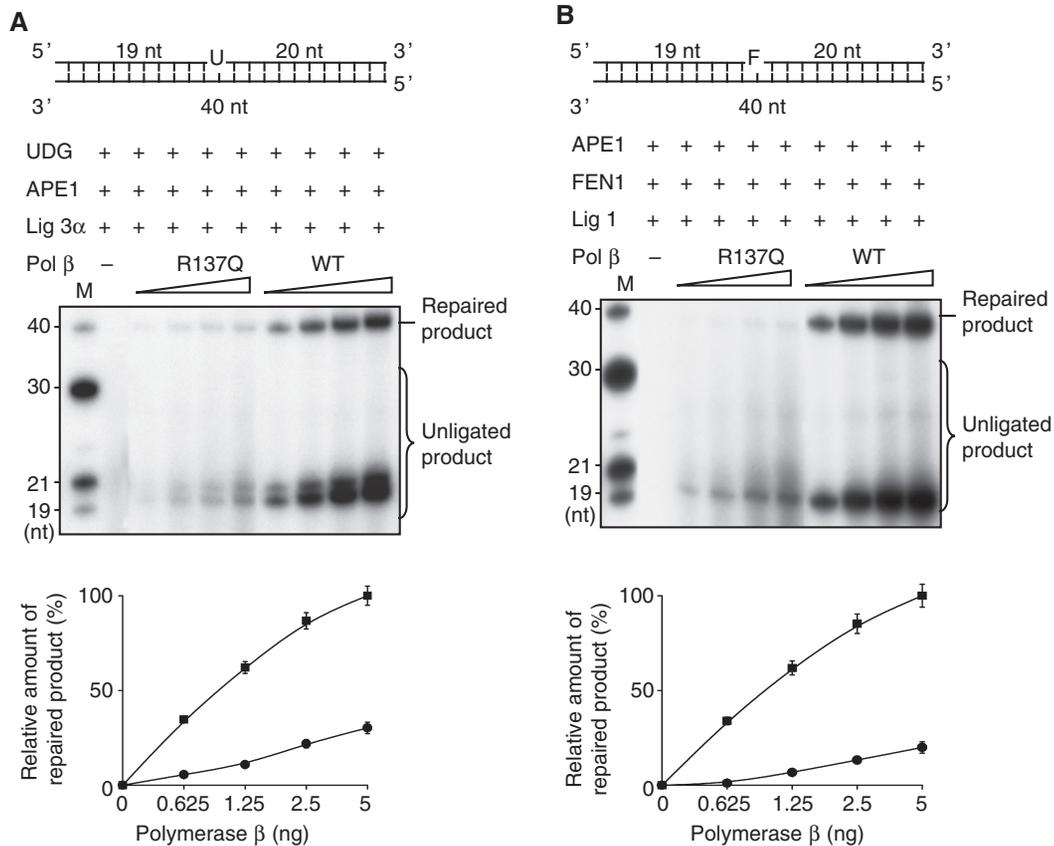
Figure 4. R137Q significantly reduces BER efficiency. (**A**) SP-BER reconstitution with purified WT and R137Q Pol β. (**B**) LP-BER reconstitution with purified WT and R137Q. The top part of each panel shows the schematic structures of the corresponding DNA substrates. The middle shows PAGE-separated products and the bottom the relative percentage of repaired product obtained with the indicated amounts of Pol β. Values represent mean ± SD of three independent assays. WT, filled squares; R137, filled circle.

The amino acid residue Arg137 can be very important for Pol β function. According to the crystal structure, R137 maps to the catalytic domain of Pol β (20). At this site, R137 is required to form hydrogen bonds with other adjacent amino acid residuals. The substitution of R137Q might disrupt the formation of hydrogen bonds and thus impair polymerase activity. Consistent with this speculation, R137Q has ∼30% of the DNA polymerase activity of WT pol β (Figure 1A and B).

Arg137 also serves as a protein posttranslational modification site. Recent research by Hottiger and coworkers (39) found that Arg137 of Pol β was methylated by protein arginine transferase 1 (PRMT1). Contrary to Hottiger's work suggesting Pol β methylation at R137 did not affect Pol β polymerase activity, our data indicated that R137Q substitution greatly reduced polymerase activity. Disparities in the two studies may have been due to differences in the properties between glutamine and a methylated arginine. Methylation of arginine increases molecular size and hydrophobicity but keeps the positive charge intact, while the arginine to glutamine substitution abolishes the positive arginine charge (50).

Our work shows that Arg137 is a regulatory site for PCNA interaction (Figure 3). PCNA, through its direct interaction with a broad range of other cellular proteins, plays essential roles in many aspects of DNA metabolism

in mammalian cells (51). PCNA was reported to enhance the LP-BER by stimulating FEN1 activity (52). Based on the polymerase used, the LP-BER can be categorized into Pol β-independent pathway (13), where Pol δ/ε are involved, and Pol β-dependent pathway, where Pol β is the only polymerase (14). PCNA was once thought only to be involved in the Pol β-independent LP-BER (13). However, recently Pol β has been shown to interact with PCNA (26), implying that PCNA may be also involved in Pol β-dependent BER. In the current study, the evaluation of the specific physiological significance of impairment of the interaction between PCNA and R137Q Pol β was complicated by the polymerase activity defect. However, we could imagine that PCNA may regulate BER by facilitating Pol β's recruitment to DNA/protein complexes formed during BER (39). It is worth to point out that R137 is also a methylation site of Pol β. A very recent study showed that Pol β can be methylated by protein arginine methyltransferase 1 at R137 site (39). Consistent with our result, methylation at R137 also blocks the interaction between Pol β and PCNA. Moreover, according to Hottiger and coworkers (39), methylation at R137 site of Pol β regulates BER efficiency. However, cells carrying the R137Q polymorphism cannot be methylated at the R137 site anymore, resulting in a loss of the BER regulatory mechanism. In living cells, the R137Q polymorphism
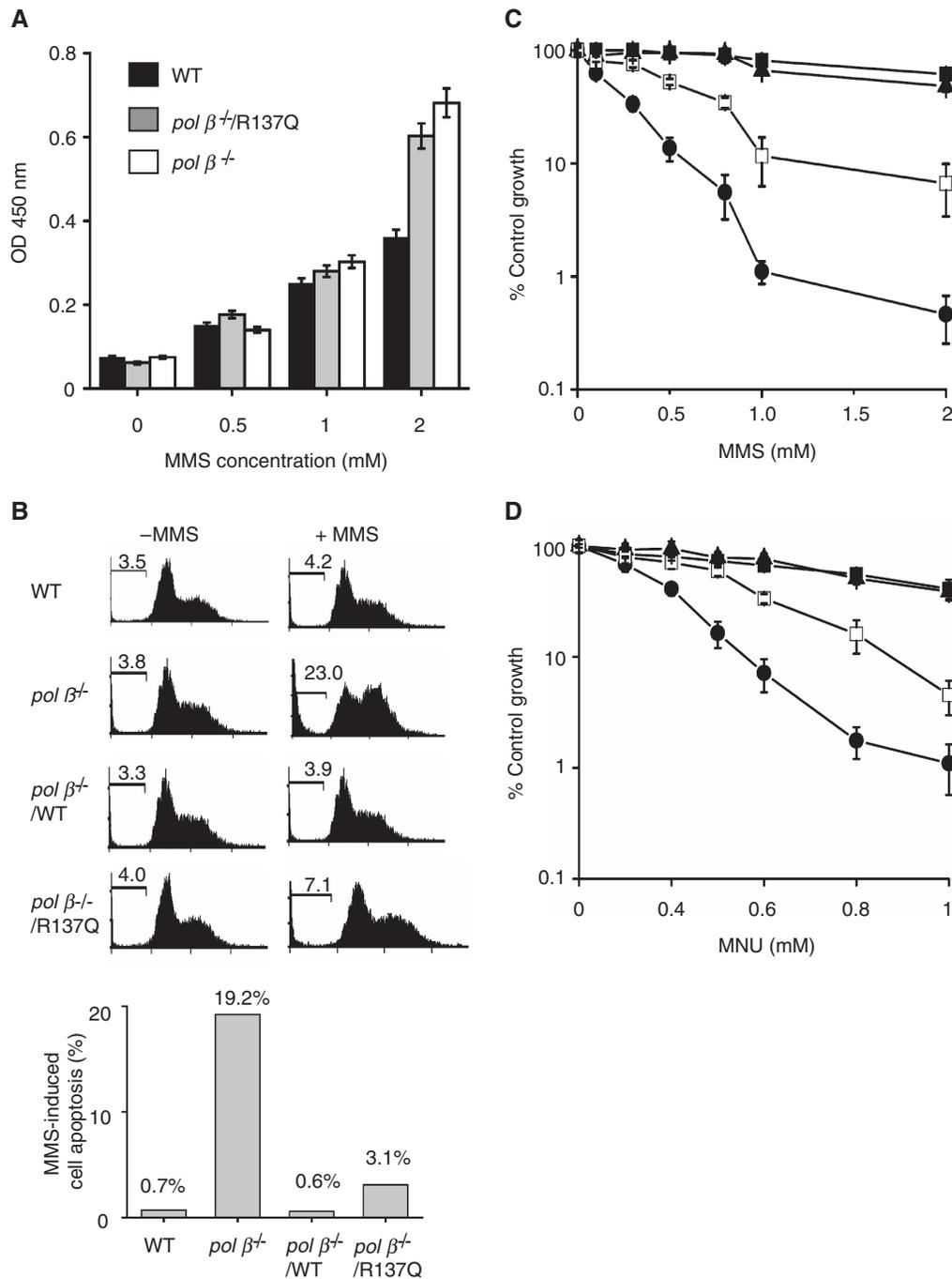
Figure 5. Polymorphic Pol β R137Q is defective in BER reconstitution using whole cell extract. (**A**) Western blotting shows the establishment of cell lines that express the Pol β WT protein or polymorphic Pol β R137Q in *pol β$^{-/-}$* MEF cells. Amount of Pol β protein was detected in 100 μg of total cell extract. (**B**) SP-BER reconstitution with WT and R137Q. (**C**) LP-BER reconstitution with WT and R137Q. The top part of each panel shows the schematic structure of the corresponding DNA substrates. The middle shows PAGE-separated products and the bottom the relative percentage of repaired product at different enzyme concentrations, as indicated. Values represent mean ± SD of three independent assays. WT, filled squares; *pol β$^{-/-}$*/WT, filled triangles; *pol β$^{-/-}$*, filled circles; *pol β$^{-/-}$*/R137Q, open squares.

could exert its effects at different levels by impairing enzyme activity, reducing protein interaction and blocking protein posttranslational modifications, exemplifying how a subtle change in polymorphism can have significant consequences to cells.

Because DNA continuously undergoes alterations, either spontaneously or induced by endogenous and exogenous factors, highly efficient DNA repair systems are critical to remove damaged DNA lesions and to maintain genome integrity. Although the genetic effect of an individual polymorphism is generally subtle, combinations of functionally relevant polymorphisms may additively or synergistically contribute to an increased risk of human diseases, such as cancers (53). In this study, we demonstrated that one single nucleotide substitution can have profound consequences on protein function and overall phenotype of organisms. The effects of the polymorphism on proteins are multiple and occur in several segregable aspects. The effect on each aspect seems subtle, but the overall consequence can be obvious and dramatic. In this

Figure 6. Cells harboring polymorphism R137Q are sensitive to DNA damage stress. (**A**) Abasic-specific DNA damage assay. Cells were treated with MMS. The amount of damaged DNA lesions was then detected by ARP reagent (see Experimental Procedures section). (**B**) Effect of MMS on induction of apoptosis in complemented *pol β$^{-/-}$* MEFs. *Pol β$^{-/-}$* MEFs complemented with WT or the R137Q variant of Pol β were treated (1 h) with 2 mM MMS. Adherent and floating cells were harvested after 24 h and analyzed by flow cytometry. The percentage of the apoptotic cells indicated. The MMS-induced increase of apoptotic cells is shown in the bottom bar. WT, *pol β$^{-/-}$*, *pol β$^{-/-}$*/WT and *pol β$^{-/-}$*/R137Q MEF cells were treated (1 h) with MMS (**C**) and MNU (**D**) at the indicated concentrations. Cellular sensitivity was determined by growth inhibition experiments. Data represents mean ± SD of four independent experiments. WT, filled squares; *pol β$^{-/-}$*/WT, filled triangles; *pol β$^{-/-}$*, filled circles; *pol β$^{-/-}$*/R137Q, open squares.

case, the Pol β R137Q polymorphism may significantly contribute to the initiation and development of human cancers through its potentially synergistic effects of impaired polymerase activity, interaction with PCNA and necessary methylation modification.

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