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The region of XRCC1 which harbours the three most common nonsynonymous polymorphic variants, is essential for the scaffolding function of XRCC1

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Abstract

XRCC1 functions as a non-enzymatic, scaffold protein in single strand break repair (SSBR) and base excision repair (BER). Here, we examine different regions of XRCC1 for their contribution to the scaffolding functions of the protein. We found that the central BRCT1 domain is essential for recruitment of XRCC1 to sites of DNA damage and DNA replication. Also, we found that ectopic expression of the region from residue 166 to 436 partially rescued the methyl methanesulfonate (MMS) hypersensitivity of XRCC1-deficient EM9 cells, suggesting a key role for this region in mediating DNA repair. The three most common amino acid variants of XRCC1, Arg194Trp, Arg280His and Arg399Gln, are located within the region comprising the NLS and BRCT1 domains, and these variants may be associated with increased incidence of specific types of cancer. While we could not detect differences in the intra-nuclear localization or the ability to support recruitment of POL β or PNKP to micro-irradiated sites for these variants relative to the conservative protein, we did observe lower foci intensity after micro-irradiation and a reduced stability of the foci with the Arg280His and Arg399Gln variants, respectively. Furthermore, when challenged with MMS or hydrogen peroxide, we detected small but consistent differences in the repair profiles of cells expressing these two variants in comparison to the conservative protein.

Keywords

XRCC1 Arg194Trp; XRCC1 Arg280His; XRCC1 Arg399Gln; DNA repair complexes; micro-irradiation

Introduction

The human genome is constantly exposed to DNA damaging agents that generate abasic sites (AP sites), base damage of different types, and single strand breaks (SSBs). Repair of

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such lesions involves base excision repair (BER) and single strand break repair (SSBR) pathways. These pathways converge into a common pathway after the initial step of base removal by a DNA glycosylase in BER [1]. X-ray repair cross-complementing protein 1 (XRCC1) has an important role in SSBR/BER by acting as a non-enzymatic, scaffold protein [2-5]. The human *XRCC1* gene was identified by complementation of Chinese hamster ovary (CHO) cells that displayed increased sensitivity to X-rays and other DNA damaging agents, particularly those that generate SSBs and base lesions [6, 7]. These cells specifically displayed reduced SSBR capacity and an increased frequency of sister chromatid exchange [8]. Recently, XRCC1 deficient cells were found to have reduced initial repair of uracil in DNA as well [2]. Notably, inefficient XRCC1 associated SSBR is reported to contribute to neurodegenerative disease in humans [4, 9].

XRCC1 interacts with a number of proteins important to the SSBR/BER pathways, including the DNA glycosylases OGG1, NEIL2, NTH1, MPG, and UNG2 [2, 10, 11], AP endonuclease-1 (APE-1) [12], DNA polymerase β (POL β) [13], DNA ligase Lig III α [14], proliferating cell nuclear antigen (PCNA) [15], poly (ADP-ribose) polymerase 1 (PARP-1) [16], PARP-2 [17] and polynucleotide kinase/phosphatase (PNKP) [18]. XRCC1 is also an important complex partner for aprataxin and tyrosyl-DNA phosphodiesterase (TDPI), two proteins found to be deficient in hereditary spinocerebellar ataxias [4, 19, 20]. XRCC1 forms dimers and oligomers that may serve as a platform for higher order complexes, and is found in complexes of significantly different sizes and composition [2, 3, 15, 21]. Recently, we proposed a model whereby XRCC1 orchestrates SSBR/BER during S phase based on characterization of complexes isolated from S phase cells [2].

XRCC1 possesses two BRCA1 carboxyl-terminal (BRCT) phospho-protein interaction domains, BRCT1 and BRCT2. Based on in vitro data, the interaction between two XRCC1 molecules is suggested to be mediated by the central BRCT1 domain [21]. Furthermore, a functional BRCT1 domain in XRCC1 is shown to be important for SSBR during both G1 and S/G2 phases of the cell cycle and for cell survival following treatment with the alkylating agent methyl methanesulfonate (MMS) [22, 23]. It is not clear whether the C-terminal BRCT2 domain contributes to complex formation between XRCC1 monomers; however, the stable interaction between XRCC1 and Lig III α , which is required ligase, is facilitated through this domain [14]. The BRCT2 domain is also involved in the XRCC1/Lig III α heterodimer formation [24]. Interestingly, recent data shows that Lig III α is dispensable for nuclear DNA repair, suggesting that XRCC1 dependent DNA repair may utilize other DNA ligases such as Lig I [25, 26].

Of the reported promoter or coding single nucleotide polymorphisms (SNPs) in human *XRCC1* within the Ensembl database*, four have been extensively studied in relation with different types of cancer. These are: -77T>C (promoter, rs3213245), Arg194Trp (R194W, rs1799782), Arg280His (R280H, rs25489) and Arg399Gln (R399Q, rs25487). The three polymorphisms found in the coding region, R194W, R280H and R399Q, have been analysed in meta-studies for different cancer types, but so far the meta-studies have failed to provide an unambiguous relationship between the polymorphism and disease prevalence [27-30]. This could partly be a result of population dependent differences in the frequency and distribution of the SNPs, e.g. the R280H variant is more frequent in Asians (7-15%) than in Caucasians (4-9%). R280H has been reported to be associated with an increased rate of breast cancer in Asians (Odds Ratio (OR) = 2.27), but not in Caucasians [31]. Another meta-study suggests that a homozygous state in R194W increases lung cancer risk, especially in Asians, while R194W heterozygosity decreases the cancer risk of lung cancer, especially in Caucasians [32]. When assessing the cumulative evidence by means of the Venice criteria,

*http://www.ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?g=ENSG00000073050.

only the -77T>C promoter polymorphism correlated with an increased risk of lung cancer at level B (moderate amount of evidence) [33]. Thus, despite the multiple reports of correlations for the different SNPs and certain cancer types, a broad consensus has proven to be elusive.

In this study we have explored different regions of XRCC1 as well as different XRCC1 SNP variants in facilitating: 1) the recruitment of XRCC1 to sites of micro-irradiation, 2) direct XRCC1-XRCC1 interaction 3) the assembly of BER proteins at regions of micro-irradiation, 4) the localization of XRCC1 to replication foci, and 4) the repair of hydrogen peroxide (H₂O₂) and MMS induced DNA damage. We found that the BRCT1 domain is key to correct intra-nuclear localization of XRCC1 to both repair and replication foci, and sufficient for a direct XRCC1-XRCC1 interaction. Furthermore, we show that the region covering the nuclear localization signal (NLS) and the BRCT1 domain (i.e. residues 166 to 436), is able to partially complement the repair defect of XRCC1-deficient cells after MMS treatment, but not after H₂O₂ treatment. For the three XRCC1 nonsynonymous SNP variants, we could not detect any major differences in intra-nuclear localization, but did observe small differences in the repair profiles and recruitment to and dissociation from micro-irradiated regions.

Materials and methods

Fluorescently tagged protein constructs

pCFP-PCNA, pXRCC1-YFP, pYFP-XRCC1, pYFP-POL β and pYFP-PNKP have been previously described [2, 15, 34]. To generate N-terminal YFP-tagged deletion fragments of XRCC1, segments of the XRCC1 coding region were PCR amplified and subcloned into the pYFP-C1 vector (Clontech). In brief, XNTD was cloned into the BglII site, MD into the BglII/BamHI site and BLB into the BglII/EcoRI site (for details see [15]). CFP versions of YFP-tagged fusion proteins were prepared by switching the AgeI/NotI fragment of the CFP and YFP-N1 vectors. SNPs of XRCC1; R194W, R280H and R399Q were introduced into pXRCC1-YFP by site-directed mutagenesis according to the protocol provided by the manufacturer (Stratagene) and verified by sequencing.

Cell lines

CHO EM9 (Xrcc1-deficient) cells stably expressing tagged human XRCC1 or the indicated deletion mutants were prepared by transfection (Fugene 6). Cells used in confocal imagery were cultured 16-48 h in normal media prior to use (Alpha modified MEM (Sigma-Aldrich) supplemented with 10% FCS, 250 μ g/ml amphotericin B (Sigma-Aldrich), 100 μ g/ml gentamycin (Invitrogen) and 1mM glutamine (BioWhittaker)). Cells used in Comet analysis were selected for stable expression by prolonged culture in normal media supplemented with Genticine (G 418, Invitrogen), followed by cell sorting and cloning by dilution. All cells were cultured at 37 °C in a 5% carbon dioxide-humidified atmosphere. The stable cells lines were used in Comet analysis. Confocal analysis (co-localisation, micro-irradiation and FRET analysis) were done 16-48 h after transient transfection of CHO EM9 cells.

Preparation of cell extracts and immunoprecipitation

Whole cell extracts were prepared by carefully resuspending the harvested cell pellet in 3 $\times$ PCV (packed cell volume) in buffer I (20 mM HEPES-KOH pH 7.8, 100 mM KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 20% glycerol, 0.5% NP-40, 1 mM DTT, 1 $\times$ Complete protease inhibitor (Roche), and phosphatase inhibitor cocktail (PIC I and II; Sigma-Aldrich)). Two μ l Omnicleave Endonuclease (200 U/ μ l; Epicenter Technologies) was added before sonication. The extracts were treated with DNase/RNase (cocktail of 2 μ l Omnicleave Endonuclease, 1 μ l DNase (10 U/ μ l; Roche), 1 μ l benzonase (250 U/ μ l; EMD), 1 μ l micrococcal nuclease

(100-300 U/mg; Sigma-Aldrich), and 10 μ l RNase (2 mg/ml; Sigma-Aldrich) per 30 mg cell extract incubated at 37 °C for 1 h), and dialysed in buffer II (20 mM HEPES-KOH pH 7.8, 100 mM KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 10% glycerol, 1 mM DTT, and 0.01 $\times$ Complete protease inhibitor) at 4 °C for a minimum of 4 h, followed by clearance by centrifugation. XRCC1-YFP and deletion constructs were immunoprecipitated (IPed) using paramagnetic Protein-A beads (Dynal) covalently coupled to an in-house polyclonal rabbit anti-GFP antibody. The beads were incubated with cell extract for a minimum of 4h at 4 °C in buffer II with additional Complete protease inhibitor (1 $\times$). The beads were washed in 10 mM Tris-HCl pH 7.5, 50 mM KCl and used in Western blotting analysis (WB). The expression levels of conservative (a.k.a. wild-type) and XRCC1 SNP variants were analysed directly by WB without IP.

Western Analysis

The membranes were blocked in 5% low fat dry milk in PBST (PBS with 0.1% Tween 20), incubated with polyclonal anti-XRCC1 (after IP, deletion mutants) (Santa Cruz sc-11429) or mouse monoclonal anti-XRCC1 (Abcam ab-1838) (cell extracts of full length XRCC1 and SNP variants) in 1% dry milk at 4 °C overnight, and incubated 1h in 1% dry milk with complementary HRP conjugated secondary antibodies and visualised using a Kodak Image Station 2000R.

Confocal imaging

Fluorescent images were acquired using a Zeiss LSM 510 Meta laser scanning microscope equipped with a Plan-Apochromate 63x/1.4 oil immersion objective. The images of live cells were acquired in growth medium, with the stage heated to 37 °C. CFP was excited at λ = 458 nm and -500 nm and detected at λ = 530 -600 nm, using consecutive scans. The thickness of the slice was 1 μ m. No image processing except contrast and intensity adjustments were performed

405 nm micro-irradiation

A Zeiss 405 nm diode laser was focused through a Plan-Apochromate 63x/1.4 oil immersion objective to a diffraction-limited spot size in a Zeiss LSM 510 Meta laser scanning microscope. The 405 nm diode output was measured to 30 mW using a FieldMaster GS energy meter (Coherent Inc.) with a low power probe. We used 60 laser beam iterations (60 it) at a speed of 1.27 μ sec/pixel over a 50 $\times$ 2 pixel area in the cell nucleus outside nucleoli (low dose, recruits POL β and PNKP, but not PCNA [3]), or 600 it (high dose, which recruits PCNA and FEN-1 as well). The high dose may give low levels of double-strand breaks (DSBs) as determined by staining for γ H2AX [3]. Time lapse image acquisition started 1 scan prior to the micro-irradiation. Signal intensities were measured using the LSM 510 Meta operating software version 4.2. The relative signal strength of the foci were obtained by dividing average foci signal strength with average signal strength measured in a non-irradiated, equally sized region of the nucleus. Only cells with similar signal intensities were analyzed. Size bars on the image equals 5 μ m.

Fluorescent resonance energy transfer (FRET) measurements

FRET was measured using the sensitized emission method as previously described [35]. Briefly, N_{FRET} was calculated from mean intensities (I) within a region of interest (ROI) containing more than 75 pixels where all pixels had intensities below 250. Channel 1 (CFP) and 3 (YFP) were measured as described for imaging, and channel 530-600 nm.

Single cell gel electrophoresis (Comet) assay

Exponentially growing CHO EM9 cells stably expressing XRCC1-YFP were treated with H₂O₂ (Sigma-Aldrich) or MMS (Sigma-Aldrich) in PBS for the indicated times and at the indicated concentrations, washed twice with PBS, and harvested in 4 °C cell medium. After centrifugation at 400 × g for 5 min, the cells were suspended in 37 °C 1% low-melt agar, mounted on a microscope slide, and immediately cooled to 4 °C. The embedded cells were lysed overnight at 4 °C in lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris-HCl, 10% DMSO, 1% Triton X-100, 17 mM NaLauroyl Sarcosine, pH 10). Alkaline (pH 13.3) single-cell gel electrophoresis was performed as described [36] except the samples were not treated with uracil DNA-glycosylase. Hundred Comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology).

Results

The BRCT1 domain is sufficient for recruitment of XRCC1 to sites of DNA damage

The contribution of the different domains of XRCC1 for the relocation of the protein itself, as well as for its binding partners, has not been extensively explored. To examine this issue, we transfected CHO cells deficient in Xrcc1 (CHO EM9) with constructs expressing either full length XRCC1 or an XRCC1 deletion mutant tagged with YFP, and determined the region responsible for XRCC1 recruitment to sites of micro-irradiation-induced DNA damage. Fig. 1A shows a schematic of the deletion mutants and the binding regions for some key SSBR/BER proteins. The XRCC1 deletion constructs are partly overlapping and contain one or more domains of XRCC1. We micro-irradiated the cells with energies sufficient for recruitment of POLβ and PNKP, but not PCNA, to sites of irradiation; the dose of micro-irradiation applied was previously shown to not introduce DSBs [3].

All constructs except BLB were strictly localized in the nuclei, consistent with those residues (amino acids 239-266) predicted to make up the NLS [16] (Fig. 1B). Full length XRCC1 rapidly assembled into foci after micro-irradiation, showing a maximum intensity increase of approximately 2.0 fold around 100 s post irradiation. MD and BLB deletion mutants were also recruited to micro-irradiated regions, while XNTD did not move into foci even at 10-100 fold higher laser doses (Fig. 1B, marked with white arrows, and data not shown). The results above were the same for all XRCC1 deletion mutants regardless of whether the YFP tag was positioned at the N- or C-terminus (not shown). Although the total nuclear intensity of the YFP-BLB deletion mutant was comparable to YFP-MD and XRCC1-YFP, the BLB mutant foci had lower intensity; we observed a maximum of a 1.4 fold increase compared to the 2 fold increase for the full length XRCC1 and the MD deletion mutant (Fig. 1B, right panel). This result suggests that residues 166-310 are important not only for nuclear localization, but also for recruitment to and/or stability of complexes at the sites of DNA damage. The importance of the BRCT1 domain for the ability of XRCC1 to form repair foci was demonstrated as the XNTD mutant did not recruit to sites of high dose micro-irradiation.

Visual colocalization of proteins does not necessarily mean that they are directly interacting, while positive fluorescence resonance energy transfer (FRET) requires that the fluorescent tags are less than 100 Å apart [37]. In order to examine whether only BRCT1, or both BRCT-domains in XRCC1 is important for the direct XRCC1-XRCC1 interactions, we measured FRET between CFP and YFP tagged MD-constructs and compared to the full length XRCC1. FRET data showed that similar to full length XRCC1s [15], the MD constructs were closer than 100 Å (Fig. 1C). This strongly suggests that the BRCT1 domain is capable of mediating the XRCC1-XRCC1 interaction.

Recruitment of DNA POL β to micro-irradiated regions requires both the NTD and the BRCT1 domain, while recruitment of PCNA and PNKP is only partly affected by XRCC1

Localization of POL β to irradiated regions is strictly dependent upon XRCC1, while recruitment of PNKP and PCNA is enhanced by XRCC1 [3]. We examined if the XRCC1 mediated recruitment of POL β , PCNA and PNKP to micro-irradiated regions was dependent upon direct interaction to their respective binding regions (Fig. 1A), which previously have been mapped by in vitro analysis of purified proteins [13, 15, 18]. CHO EM9 cells were either transfected with tagged POL β , PCNA, or PNKP alone or together with tagged full length XRCC1, MD, or BLB (CPF/YFP in pair). Because XNTD did not form foci after micro-irradiation (Fig. 1B), it was not included in these studies. Confocal analysis verified that even upon very high doses of laser micro-irradiation (1200 iterations), more than sufficient to introduce DNA DSBs, no POL β foci could be detected in absence of XRCC1, while PNKP and PCNA were recruited to sites of damage (Table 1). POL β was recruited to micro-irradiated regions when co-expressed with full length XRCC1, but not with the MD or BLB fragments (Table I). Thus, the reported NTD binding region in XRCC1 is required for relocation of POL β . Recruitment of PCNA to micro-irradiated regions increased in the presence of XRCC1, and this may be associated with a direct interaction between XRCC1 and PCNA, as MD, but not BLB, enhanced the recruitment of PCNA to micro-irradiated regions (Table I). Furthermore, we found that BLB, but not MD, increased localization of PNKP to sites of DNA damage generated by micro-irradiation, consistent with the direct interaction between PNKP and XRCC1 facilitating the recruitment (Table I). CFP-tagged POL β , PCNA, and PNKP were all recruited similarly to micro-irradiated regions in the parental XRCC1 proficient cell line (CHO AA8) used as a control (Table I). Images corresponding to Table 1 are given in Supplemental Fig. S1.

The BRCT1 domain of XRCC1 is required for recruitment of XRCC1 to replication foci

Although XRCC1 lacks the two reported PCNA interaction motifs [35, 38], XRCC1 interacts directly with PCNA and localizes to replication foci independent of an exogenous source of DNA damage [15]. XRCC1 likely operates here as part of a distinct replication associated complex proficient in BER [2]. The results presented in Table I show that the identified PCNA binding region in XRCC1 [15] is important for recruitment of PCNA to micro-irradiated regions. We examined whether the DNA damage independent localization of XRCC1 to replication foci was directed by the PCNA binding region in XRCC1. We found that both BLB and MD, similar to full length XRCC1, colocalized with PCNA in replication foci (Fig. 2), even though only MD includes the reported PCNA binding region (Fig. 1A). XNTD, on the other hand, which includes most of the PCNA binding region and pulls down PCNA in vitro [15], did not colocalize with replication foci. Thus, colocalization of XRCC1 and PCNA in replication foci is dependent on the region between 310 and 436 of XRCC1, which includes the BRCT1 domain. In addition, co-localization of the BLB deletion mutant and PCNA implies that XRCC1 can be recruited to replication foci via binding to proteins other than PCNA. Candidate proteins are PARP-1 [39], MPG [40] and UNG2 [2, 41], which have been found in replication foci and have their XRCC1 binding domains in, or partially within, the BLB deletion mutant.

The NLS-BRCT1 region in XRCC1 is important for rapid repair of MMS induced DNA damage

XRCC1-deficient cells show an increased sensitivity to DNA damage introduced by MMS (10 fold) and H₂O₂ (2 fold) [7]. Because both MD and BLB were recruited to micro-irradiated regions and colocalized with replication foci, they could potentially scaffold repair proteins and support SSBR/BER. We examined this hypothesis in cloned cell lines of CHO EM9 cells stably expressing the deletion constructs using the Comet assay. The Comet assay as performed here enabled us to detect the total amount of DSBs, SSBs and alkali-labile

sites (i.e. AP sites); hence, all repair intermediates of BER/SSBR after removal of damaged bases.

In three independent experiments we found that the MD deletion mutant, unlike the XNTD or BLB fragment, was able to partially complement XRCC1 for repair of MMS induced DNA damage (Fig. 3A show merged results). Judged by the average fluorescence intensities and Western blot analysis (Fig. 3C), this outcome was clearly not related to a higher expression level of MD compared to the other deletion mutants. The MD mutant spans the region reported to be important for interactions with DNA glycosylases (amino acids 166-436), such as MPG [10], as well as with PCNA, APE-1, and PARP-1 [12, 15, 16]. The MD fragment, however, lacks the portions of XRCC1 responsible for the interactions with and POL β [13, 14]. Since the MD deletion mutant also contains the reported binding regions for OGG1, NTH1 and NEIL2 [10, 11], DNA glycosylases primarily involved in the repair of oxidative base lesions, we tested the ability of this fragment to complement XRCC1 after treatment of cells with H₂O₂. Neither MD, nor the other deletion mutants, contributed reproducibly to any change in DNA repair after H₂O₂ treatment in parallel experiments (Fig. 3B). Thus, our results indicate that a specific interaction of the MD deletion mutant is critical for the efficient XRCC1-directed repair of MMS-induced DNA damage, yet is dispensable for oxidative DNA damage repair. We examined whether the complementation observed may stem from an interaction between XRCC1 and MPG, but found that knock down of MPG by siRNA did not change the pattern of repair for any of the deletion constructs (data not shown).

The variants Arg194Trp, Arg280His and Arg399Gln of XRCC1 display subtle differences in repair profiles

The MD deletion mutant exhibits similar nuclear localization to full length XRCC1, responds similarly to micro-irradiation as full length XRCC1, and partly complements the MMS hypersensitivity of the EM9 cell line. The three most frequent non-synonymous SNPs found in XRCC1, Arg194Trp (R194W), Arg280His (R280H) and Arg399Gln (R399Q), are all located within the MD fragment (marked by stars in Fig. 1A). Since this region seems to be essential for both the proper localization and the scaffolding function of XRCC1, we examined intra-nuclear localization of YFP-tagged versions of these XRCC1 polymorphic variants. All three variants showed a localization pattern similar to the conservative (a.k.a. wild-type) form of XRCC1 (Fig. 4), consistent with previous results [42]. Moreover, all three variants colocalized with PCNA in replication foci (panel A), and supported relocation of POL β (panel B) and PNKP (panel C) to regions of micro-irradiation similar to conservative XRCC1. We note that only the R399Q XRCC1 variant tagged with YFP is shown in comparison with conservative XRCC1-YFP in Fig. 4; the other two polymorphic variants behaved similarly and are shown in Supplemental Fig. S2.

We next determined quantitatively the kinetics of appearance and dissociation of the polymorphic variants from sites of micro-irradiation relative to the conservative XRCC1. For the R194W variant, we did not detect a significantly different pattern of relocation compared to the conservative XRCC1 (Fig. 4D, left panel), although a tendency toward a lower foci intensity, hence less accumulation, was observed. The R399Q variant reached its highest foci intensity peak earlier than the conservative XRCC1 (after 60 s versus 120 s, respectively, shown in red), suggesting a reduced ability to stay at the site of damage. Moreover, the initial slope of the curve supported a more immediate dissociation of the R399Q variant (Fig. 4D, right panel), although the difference in average intensities was not statistically significant until 300 s (marked with *). For heterozygous R280H, a recent report [42] suggested that this variant dissociated more rapidly than its conservative form from sites of micro-irradiation in HeLa cells. Under our experimental conditions (homozygous expression and low dose micro-irradiation of CHO EM9 cells not introducing DSBs), we

could not detect any difference in the rate of dissociation, i.e. the slopes are equal for the R280H variant compared to conservative XRCC1. However, R280H did display a tendency, which was statistically significant after 300 s, for impaired foci formation, i.e. lower foci intensity (Fig. 4D, mid panel). The time to reach maximum intensity was similar for the R280H variant and the conservative XRCC1. Finally, we detected small differences in the ability of two (R194W and R399Q) of the XRCC1 variants to complex with the conservative XRCC1 by FRET analysis (Supplemental Fig. S3).

Because reduced ability to be recruited to foci or an increased rate of dissociation could lead to repair deficiencies, we next examined the repair kinetics of MMS and H₂O₂ induced DNA damage in *Xrcc1*-deficient cells complemented with the conservative or a variant XRCC1 protein using the Comet assay as described earlier. In order to detect subtle differences in repair efficacy between the conservative XRCC1 and polymorphic variants, we used high doses of MMS and H₂O₂ (8 and 4 fold, respectively, higher than in experiments shown in Fig. 3). After 120 min, the DNA damage levels were reduced for each of the complemented cell lines, indicating that all cells were able to recover after these doses of MMS and H₂O₂ (Fig. 5A). The polymorphic variants, however, showed significant and reproducible differences in the pattern of % tail DNA compared to the conservative XRCC1 after MMS treatment; initially lower tail. A disparate pattern was also seen for the R280H and R399Q variants after H₂O₂ treatment (Fig. 5A, upper row shows comet tail distribution in one representative experiment, two other experiments are shown in Supplemental Fig. S4). Western analysis (Fig. 5B) showed no correlation between the DNA repair profile and the expression level of the polymorphic variants relative to the conservative XRCC1 protein.

Discussion

Point mutations in the BRCT1 domain of XRCC1 (i.e. L1360/361DD and W285D) are reported to affect cellular resistance to MMS induced DNA damage. However, the intra-nuclear localization of the described BRCT1 mutants is not known. Since these mutations disrupt the correct folding of XRCC1, it is possible that the amino acid substitutions may in fact impair proper intra-cellular distribution [22, 23, 43]. Because expression of the BLB-mutant does not rescue the *Xrcc1*-deficient cells, our data suggest that it is not the BRCT1 domain *per se*, but the larger NLS-BRCT1 region (residue 166 to 436) that is important for the ability of XRCC1 to support repair after MMS treatment.

We have recently published that localization of POL β to sites of irradiation was completely dependent on XRCC1, while the recruitment of PNKP and PCNA was only enhanced in the presence of XRCC1 [3]. Studies have shown that XRCC1 binds to nicked and gapped DNA [44] and that the NTD domain of XRCC1 exhibits a specific affinity for these lesions [45]. This domain of XRCC1 (NTD) also interacts with POL β [13]. A previous study suggests a POL β dependent recruitment of XRCC1 to sites of DNA damage [46]. However, the fact that the XNTD deletion mutant does not form foci suggests that the interaction with POL β is not sufficient for re-localization of XRCC1 to sites of damage.

XRCC1 and the three polymorphic variants, R194W, R280H and R399Q, show similar intra-nuclear localization, and all variants supported POL β and PNKP relocation to sites of micro-irradiation. Interestingly, the R399Q variant is located within a conserved PAR-binding motif (amino acids 379-400 [47]), (see Fig. 1A). We found that this variant had a reduced ability to remain at sites of micro-irradiation, and this could possibly be due to reduced PAR-binding. Reduced XRCC1 recruitment and/or ability to form stable complexes will likely affect recruitment of XRCC1 interacting proteins and the overall efficiency of repair.

The Comet assay as performed here detects AP sites, SSB and DSBs and several forms of repair intermediates, but not damaged bases. The differences in the repair profiles of the XRCC1 variants after MMS and H₂O₂ treatment could be explained by reduced recruitment of these XRCC1s and interacting proteins to sites of DNA damage, reduced ability to make complexes or interact with DNA glycosylases, and/or reduced efficiency of excision of damaged bases or resolution of strand break intermediates. Reduced base excision will result in less % tail DNA immediately or shortly after DNA damage introduction, which is what we see (Fig. 5A). Thus, what may be interpreted as more efficient initial repair by merely looking at the comet tails, could actually be a delayed BER response as illustrated in the model in Fig. 5C (broken line). Such differences may only be detectable in a small time window and may vary with different types of DNA damage. Interestingly, NSCLC (non-small cell lung cancer) patients, who are heterozygous for the XRCC1 variants R280H and R399Q, were found to have significantly lower 8-oxoG incision activity in extracts from lung tissue compared to extracts from NSCLC patients homozygous for the conservative form of XRCC1 [48]. Because XRCC1 forms multimeric complexes, reduced ability of the variants for complex stability (Fig. 4D and Supplemental Fig. S3) could possibly affect repair also in heterozygote variants.

Recent reports concerning a possible association of XRCC1 variants with cancer incidence have detected high OR values when including several variants within the XRCC1 protein or when combined with SNPs in other DNA repair proteins. For instance, R399Q was reported to be associated with increased breast cancer risk alone (OR=4.67) and together with the -77C>T variant (OR=7.04) [49]. R399Q was furthermore found to be associated with an increased colorectal cancer risk alone (OR=1.65), while a significantly stronger association was found in combination with a XPD SNP (Lys751Asn) (subunit of transcription factor IIH, involved in nucleotide excision repair, NER) (OR=3.52) [50]. Thus, even if the differences in the repair profiles of the SNPs detected under our experimental conditions are subtle, they might be of biological significance, and perhaps more so when combined with polymorphic variants of other DNA repair proteins in the same pathway.

In summary, in this paper we have shown that the region in XRCC1 harbouring the three most common SNPs is essential for localization of XRCC1 and its interacting partners to sites of DNA damage and DNA replication. Although we did not observe profound differences in the functional activities of the XRCC1 polymorphic variants, there was a statistically significant reduction in the ability of R280H to be recruited to or R399Q to remain at foci after micro-irradiation. A significant change in repair profile after DNA damage induced by H₂O₂ and MMS was also observed with the R280H and R399Q variant-complemented EM9 cell lines. The observed differences herein may ultimately affect the overall regulation of BER/SSBR and account for the reported associations of XRCC1 polymorphisms with increased risk of disease.

Supplementary Material

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Acknowledgments

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References

- [1]. Zharkov DO. Base excision DNA repair. *Cell Mol Life Sci.* 2008; 65:1544–1565. [PubMed: 18259689]
- [2]. Akbari M, Solvang-Garten K, Hanssen-Bauer A, Lieske NV, Pettersen HS, Pettersen GK, Wilson DM 3rd, Krokan HE, Otterlei M. Direct interaction between XRCC1 and UNG2 facilitates rapid repair of uracil in DNA by XRCC1 complexes. *DNA Repair (Amst).* 2010; 9:785–795. [PubMed: 20466601]
- [3]. Hanssen-Bauer A, Solvang-Garten K, Sundheim O, Pena-Diaz J, Andersen S, Slupphaug G, Krokan HE, Wilson DM 3rd, Akbari M, Otterlei M. XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage. *Environ Mol Mutagen.* 2011
- [4]. El-Khamisy SF, Saifi GM, Weinfeld M, Johansson F, Helleday T, Lupski JR, Caldecott KW. Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. *Nature.* 2005; 434:108–113. [PubMed: 15744309]
- [5]. Luo H, Chan DW, Yang T, Rodriguez M, Chen BP, Leng M, Mu JJ, Chen D, Songyang Z, Wang Y, Qin J. A new XRCC1-containing complex and its role in cellular survival of methyl methanesulfonate treatment. *Mol. Cell Biol.* 2004; 24:8356–8365. [PubMed: 15367657]
- [6]. Thompson LH, Brookman KW, Jones NJ, Allen SA, Carrano AV. Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol. Cell Biol.* 1990; 10:6160–6171. [PubMed: 2247054]
- [7]. Thompson LH, Brookman KW, Dillehay LE, Carrano AV, Mazrimas JA, Mooney CL, Minkler JL. A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister-chromatid exchange. *Mutat. Res.* 1982; 95:427–440. [PubMed: 6889677]
- [8]. Caldecott KW. Mammalian DNA single-strand break repair: an X-ra(y)ted affair. *Bioessays.* 2001; 23:447–455. [PubMed: 11340626]
- [9]. Hirano M, Yamamoto A, Mori T, Lan L, Iwamoto TA, Aoki M, Shimada K, Furiya Y, Kariya S, Asai H, Yasui A, Nishiwaki T, Imoto K, Kobayashi N, Kiriya T, Nagata T, Konishi N, Itoyama Y, Ueno S. DNA single-strand break repair is impaired in aprataxin-related ataxia. *Ann. Neurol.* 2007; 61:162–174. [PubMed: 17315206]
- [10]. Campalans A, Marsin S, Nakabeppu Y, O'Connor R, Boiteux T,S, Radicella JP. XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. *DNA Repair (Amst.).* 2005; 4:826–835. [PubMed: 15927541]
- [11]. Marsin S, Vidal AE, Sossou M, Menissier-de Murcia J, Le Page F, Boiteux S, de Murcia G, Radicella JP. Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. *J. Biol. Chem.* 2003; 278:44068–44074. [PubMed: 12933815]
- [12]. Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J.* 2001; 20:6530–6539. [PubMed: 11707423]
- [13]. Caldecott KW, Aoufouchi S, Johnson P, Shall S. XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. *Nucleic Acids Res.* 1996; 24:4387–4394. [PubMed: 8948628]
- [14]. Caldecott KW, McKeown CK, Tucker JD, Ljungquist S, Thompson LH. An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Mol. Cell Biol.* 1994; 14:68–76. [PubMed: 8264637]
- [15]. Fan J, Otterlei M, Wong HK, Tomkinson AE, Wilson DM 3rd. XRCC1 co-localizes and physically interacts with PCNA. *Nucleic Acids Res.* 2004; 32:2193–2201. [PubMed: 15107487]
- [16]. Masson M, Niedergang C, Schreiber V, Muller S, Menissier-de Murcia J, de Murcia G. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol Cell Biol.* 1998; 18:3563–3571. [PubMed: 9584196]
- [17]. Schreiber V, Ame JC, Dolle P, Schultz I, Rinaldi B, Fraulob V, Menissier-de Murcia J, de Murcia G. Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA

- repair in association with PARP-1 and XRCC1. *J. Biol. Chem.* 2002; 277:23028–23036. [PubMed: 11948190]
- [18]. Whitehouse CJ, Taylor RM, Thistlethwaite A, Zhang H, Karimi-Busheri F, Lasko DD, Weinfeld M, Caldecott KW. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. *Cell.* 2001; 104:107–117. [PubMed: 11163244]
- [19]. Plo I, Liao ZY, Barcelo JM, Kohlhagen G, Caldecott KW, Weinfeld M, Pommier Y. Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. *DNA Repair (Amst).* 2003; 2:1087–1100. [PubMed: 13679147]
- [20]. Ahel I, Rass U, El-Khamisy SF, Katyal S, Clements PM, McKinnon PJ, Caldecott KW, West SC. The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. *Nature.* 2006; 443:713–716. [PubMed: 16964241]
- [21]. Beernink PT, Hwang M, Ramirez M, Murphy MB, Doyle SA, Thelen MP. Specificity of protein interactions mediated by BRCT domains of the XRCC1 DNA repair protein. *J. Biol. Chem.* 2005; 280:30206–30213. [PubMed: 15987676]
- [22]. Taylor RM, Thistlethwaite A, Caldecott KW. Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair. *Mol Cell Biol.* 2002; 22:2556–2563. [PubMed: 11909950]
- [23]. Kubota Y, Horiuchi S. Independent roles of XRCC1's two BRCT motifs in recovery from methylation damage. *DNA Repair (Amst).* 2003; 2:407–415. [PubMed: 12606121]
- [24]. Cuneo MJ, Gabel SA, Krahn JM, Ricker MA, London RE. The structural basis for partitioning of the XRCC1/DNA ligase III- $\{\alpha\}$ BRCT-mediated dimer complexes. *Nucleic acids research.* 2011
- [25]. Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis AK, Van Houten B, Shuman S, McKinnon PJ, Jasini M. Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. *Nature.* 2011; 471:245–248. [PubMed: 21390132]
- [26]. Gao Y, Katyal S, Lee Y, Zhao J, Rehgle JE, Russell HR, McKinnon PJ. DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair. *Nature.* 2011; 471:240–244. [PubMed: 21390131]
- [27]. Gsur A, Bernhart K, Baierl A, Feik E, Fuhrlinger G, Hofer P, Leeb G, Mach K, Micksche M. No association of XRCC1 polymorphisms Arg194Trp and Arg399Gln with colorectal cancer risk. *Cancer Epidemiol.* 2011
- [28]. Xue H, Ni P, Lin B, Xu H, Huang G. X-ray repair cross-complementing group 1 (XRCC1) genetic polymorphisms and gastric cancer risk: A HuGE review and meta-analysis. *American journal of epidemiology.* 2011; 173:363–375. [PubMed: 21216841]
- [29]. Chen B, Zhou Y, Yang P, Wu XT. Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis. *Mol Biol Rep.* 2011
- [30]. Huang J, Zhang J, Zhao Y, Liao B, Liu J, Li L, Liao M, Wang L. The Arg194Trp polymorphism in the XRCC1 gene and cancer risk in Chinese Mainland population: a meta-analysis. *Mol Biol Rep.* 2011
- [31]. Li H, Ha TC, Tai BC. XRCC1 gene polymorphisms and breast cancer risk in different populations: a meta-analysis. *Breast.* 2009; 18:183–191. [PubMed: 19446452]
- [32]. Jiang J, Liang X, Zhou X, Huang R, Chu Z, Zhan Q, Lin H. DNA repair gene X-ray repair cross complementing group 1 Arg194Trp polymorphism on the risk of lung cancer: a meta-analysis on 22 studies. *J Thorac Oncol.* 2010; 5:1741–1747. [PubMed: 20975374]
- [33]. Vineis P, Manuguerra M, Kavvoura FK, Guarrera S, Allione A, Rosa F, Di Gregorio A, Polidoro S, Saletta F, Ioannidis JP, Matullo G. A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility. *J Natl Cancer Inst.* 2009; 101:24–36. [PubMed: 19116388]
- [34]. Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, Akbari M, Sundheim O, Bjoras M, Slupphaug G, Seeberg E, Krokan HE. Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature.* 2003; 421:859–863. [PubMed: 12594517]
- [35]. Gilljam KM, Feyzi E, Aas PA, Sousa MM, Muller R, Vagbo CB, Catterall TC, Liabakk NB, Slupphaug G, Drablos F, Krokan HE, Otterlei M. Identification of a novel, widespread, and

- functionally important PCNA-binding motif. *J Cell Biol.* 2009; 186:645–654. [PubMed: 19736315]
- [36]. Nilsen H, Rosewell I, Robins P, Skjelbred CF, Andersen S, Slupphaug G, Daly G, Krokan HE, Lindahl T, Barnes DE. Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication. *Mol. Cell.* 2000; 5:1059–1065. [PubMed: 10912000]
- [37]. Matyus L. Fluorescence resonance energy transfer measurements on cell surfaces. A spectroscopic tool for determining protein interactions. *J Photochem Photobiol B.* 1992; 12:323–337. [PubMed: 1578295]
- [38]. Warbrick E. The puzzle of PCNA's many partners. *Bioessays.* 2000; 22:997–1006. [PubMed: 11056476]
- [39]. Bryant HE, Petermann E, Schultz N, Jemth AS, Loseva O, Issaeva N, Johansson F, Fernandez S, McGlynn P, Helleday T. PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. *EMBO J.* 2009; 28:2601–2615. [PubMed: 19629035]
- [40]. Xia L, Zheng L, Lee HW, Bates SE, Federico L, Shen B, O'Connor TR. Human 3-methyladenine-DNA glycosylase: effect of sequence context on excision, association with PCNA, and stimulation by AP endonuclease. *J. Mol. Biol.* 2005; 346:1259–1274. [PubMed: 15713479]
- [41]. Otterlei M, Warbrick E, Nagelhus TA, Haug T, Slupphaug G, Akbari M, Aas PA, Steinsbekk K, Bakke O, Krokan HE. Post-replicative base excision repair in replication foci. *EMBO J.* 1999; 18:3834–3844. [PubMed: 10393198]
- [42]. Berquist BR, Singh DK, Fan J, Kim D, Gillenwater E, Kulkarni A, Bohr VA, Ackerman EJ, Tomkinson AE, Wilson DM 3rd. Functional capacity of XRCC1 protein variants identified in DNA repair-deficient Chinese hamster ovary cell lines and the human population. *Nucleic Acids Res.* 2010; 38:5023–5035. [PubMed: 20385586]
- [43]. Taylor RM, Moore DJ, Whitehouse J, Johnson P, Caldecott KW. A cell cycle-specific requirement for the XRCC1 BRCT II domain during mammalian DNA strand break repair. *Mol Cell Biol.* 2000; 20:735–740. [PubMed: 10611252]
- [44]. Mani RS, Karimi-Busheri F, Fanta M, Caldecott KW, Cass CE, Weinfeld M. Biophysical characterization of human XRCC1 and its binding to damaged and undamaged DNA. *Biochemistry.* 2004; 43:16505–16514. [PubMed: 15610045]
- [45]. Marintchev A, Robertson A, Dimitriadis EK, Prasad R, Wilson SH, Mullen GP. Domain specific interaction in the XRCC1-DNA polymerase beta complex. *Nucleic acids research.* 2000; 28:2049–2059. [PubMed: 10773072]
- [46]. Parsons JL, Dianova II, Allinson SL, Dianov GL. DNA polymerase beta promotes recruitment of DNA ligase III α -XRCC1 to sites of base excision repair. *Biochemistry.* 2005; 44:10613–10619. [PubMed: 16060670]
- [47]. Stilmann M, Hinz M, Arslan SC, Zimmer A, Schreiber V, Scheidereit C. A nuclear poly(ADP-ribose)-dependent signalosome confers DNA damage-induced IkappaB kinase activation. *Mol Cell.* 2009; 36:365–378. [PubMed: 19917246]
- [48]. Swoboda M, Fordell T, Klunder K, Dahlstrom JM, Miranda M, Buth C, Schafer KJ, Mauritsson J, L'Huillier A, Gisselbrecht M. Phase measurement of resonant two-photon ionization in helium. *Phys Rev Lett.* 2010; 104:103003. [PubMed: 20366419]
- [49]. Sterpone S, Mastellone V, Padua L, Novelli F, Patrono C, Cornetta T, Giammarino D, Donato V, Testa A, Cozzi R. Single-nucleotide polymorphisms in BER and HRR genes, XRCC1 haplotypes and breast cancer risk in Caucasian women. *Journal of cancer research and clinical oncology.* 2010; 136:631–636. [PubMed: 20140625]
- [50]. Wang J, Zhao Y, Jiang J, Gajalakshmi V, Kuriki K, Nakamura S, Akasaka S, Ishikawa H, Suzuki S, Nagaya T, Tokudome S. Polymorphisms in DNA repair genes XRCC1, XRCC3 and XPD, and colorectal cancer risk: a case-control study in an Indian population. *Journal of cancer research and clinical oncology.* 2010; 136:1517–1525. [PubMed: 20229274]

1. The BRCT1 region in XRCC1 is vital for complex formation, recruitment to DNA damage, correct intra-nuclear localisation and DNA repair
2. The R194W, R280H and R399Q SNP variants of XRCC1 show normal intra-nuclear localisation.
3. R280H and R399Q SNP variants of XRCC1 have reduced ability to be recruited to, or stay at, sites of DNA damage
4. The R194W, R280H and R399Q SNP variants of XRCC1 show small, but consistent differences in the repair profiles after H₂O₂ and MMS challenge

Fig. 1. Cellular localization and level of recruitment of XRCC1 deletion mutants to micro-irradiated regions

(A) Overview of the distinct domains in XRCC1, sites of interactions with other proteins, position of the three most common nonsynonymous polymorphic variants and definition of deletion mutants used in this study. XNTD: extended XRCC1 N-terminal Domain, BRCT1/2: BRCA1 C-terminus (BRCT) domains. NLS: nuclear localisation domain. BLB: BRCT1 Linked BRCT2 domain, MD: Mid Domain (B) Left panel: Recruitment of full length XRCC1-YFP and YFP-XNTD, YFP-MD, YFP-BLB deletion mutants to sites of micro-irradiation (60 it, see material and methods). Bars; 5 μm. Arrows indicate irradiated regions. Right panel: Graphs showing fold increase in foci intensities after micro-irradiation of the irradiated region. Mean ± SEM, XRCC1 n=15, XNTD n=5, MD n=15, BLB n=20. (C) N_{FRET} is calculated by sensitized emission as described in materials and methods. Representative data from one out of three independent experiments are shown. Cells were co-transfected with CFP and YFP vectors (CFP/YFP), full length (XRCC1-CFP/XRCC1-YFP) and MD deletion mutants (CFP-MD/YFP-MD). N_{FRET} in selected regions of interest (ROI) are presented as dots with mean ± SEM, n=30-36.

Fig. 2. Colocalization of the XRCC1 deletion mutants and PCNA in replication foci
 Confocal fluorescence images of XRCC1 and its deletion mutants with YFP-tag (green) co-expressed with CFP-tagged PCNA (red) in live S phase CHO EM9 cells. The yellow dots in the merged pictures (third row) indicate colocalization of the proteins. Bars; 5 μ m.

Fig. 3. Comet analysis of the XRCC1 deletion mutants for their ability to complement full length XRCC1

Analysis of CHO EM9 cells stably expressing YFP, XRCC1-YFP, YFP-XNTD, YFP-MD and YFP-BLB. Comet analysis after 10 min treatment with (A) MMS (600 μ M) or (B) H₂O₂ (62.5 μ M). 100 comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology). Data presented are merged from three independent experiments (n=100), and are presented as scatter plot of % Tail DNA on the y-axis. Mean $\pm$ SEM, n=300. *p<0.0001. (C) Expression levels of the fusion proteins in the different cell lines detected after immunoprecipitation (IP). Two bands are seen due to partial degradation of the fusion proteins. The band at 50 kDa represent IgG from the IP.

Fig. 4. Colocalization analysis of XRCC1 or XRCC1 SNP variants, with PNKP in micro-irradiated regions, and with PCNA in replication foci

(A) CHO EM9 cells co-transfected with conservative XRCC1-YFP (left panel) and XRCC1_{R399Q}-YFP (right panel, R399Q is representative for all three variants) with CFP-PCNA, (B) conservative XRCC1-YFP (left panel) and XRCC1_{R399Q}-YFP (right panel, R399Q is representative for all three variants) with CFP-POLβ (C) conservative XRCC1-YFP (left panel) and XRCC1_{R399Q}-YFP (right panel, R399Q is representative for all three variants) with CFP-PNKP. Bars 5 μm. (D) Graphs showing recruitment and dissociation of XRCC1-YFP and XRCC1_{R194W}-YFP (left panel), XRCC1_{R280H}-YFP (mid panel) and XRCC1_{R399Q}-YFP (right panel) to selected micro-irradiated regions (60 it, see material and methods). Graphs show mean±SEM, conservative XRCC1-YFP, n=28 (from three independent experiments), XRCC1_{R194W}-YFP, n=10, XRCC1_{R280H}-YFP, n=14 and XRCC1_{R399Q}-YFP (in red), n=14. * p<0.05.

Fig. 5. Analysis of the ability of the XRCC1 SNP variants to complement conservative XRCC1 (A) Comet analysis of CHO EM9 cells stably expressing XRCC1-YFP, XRCC1R194W-YFP, XRCC1R280H-YFP, XRCC1R399Q-YFP after treatment with MMS (4.8 mM) (left panel) and H₂O₂ (250 μ M) (right panel) after 0 and 120 min recovery. 100 comets were selected randomly from each slide and evaluated using Komet 5.0 Imaging Software (Andor Technology). Data presented is from one representative out of three independent experiments, and is presented as scatter plot of % tail DNA on the y-axis. Mean $\pm$ SEM, n=100 (B) Expression levels of the fusion proteins in the corresponding cell lines (C) Model of different repair kinetics between conservative and polymorphic variants. Dashed grey line represents polymorphic variants with delayed BER.

Table 1
Ability of XRCC1 and XRCC1 deletion mutants to support foci formation after micro-irradiation

Cell lines	Cells transfected with indicated constructs		<i>POLβ</i>	<i>PCNA</i>	<i>PNKP</i>
EM9 (Xrcc1 ^{-/-})			- 1	1	1
AA8			+++ 1	+++ 1	+++ 1
EM9 (Xrcc1 ^{-/-})		<i>f.l. XRCC1</i>	+++ 1	+++ 1	+++ 1
EM9 (Xrcc1 ^{-/-})		<i>XNTD</i>	N.D.	N.D.	N.D.
EM9 (Xrcc1 ^{-/-})		<i>MD</i>	-	++	+
EM9 (Xrcc1 ^{-/-})		<i>BLB</i>	-	+	++

1 Published previously in Hanssen-Bauer et al., 2011.
N.D. = Not determined
f.l.= full length
- = no foci
+ = foci after 1200 iterations
++ = foci after 600 iterations
+++ = foci after 60 iterations