DNA Repair 40 (2016) 67–76

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

The role of HERC2 and RNF8 ubiquitin E3 ligases in the promotion of translesion DNA synthesis in the chicken DT40 cell line

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 12 January 2016
Accepted 3 February 2016
Available online 2 March 2016

Keywords:
HERC2
RNF8
Translesion DNA synthesis
Post-replication repair
Ubiquitination

ABSTRACT

The replicative DNA polymerases are generally blocked by template DNA damage. The resulting replication arrest can be released by one of two post-replication repair (PRR) pathways, translesion DNA synthesis (TLS) and template switching by homologous recombination (HR). The HERC2 ubiquitin ligase plays a role in homologous recombination by facilitating the assembly of the Ubc13 ubiquitin-conjugating enzyme with the RNF8 ubiquitin ligase. To explore the role of HERC2 and RNF8 in PRR, we examined immunoglobulin diversification in chicken DT40 cells deficient in HERC2 and RNF8. Unexpectedly, the *HERC2*$^{-/-}$ and *RNF8*$^{-/-}$ cells and *HERC2*$^{-/-}$/*RNF8*$^{-/-}$ double mutant cells exhibit a significant reduction in the rate of immunoglobulin (Ig) hypermutation, compared to *wild-type* cells. Further, the *HERC2*$^{-/-}$ and *RNF8*$^{-/-}$ mutants exhibit defective maintenance of replication fork progression immediately after exposure to UV while retaining proficient post-replicative gap filling. These mutants are both proficient in mono-ubiquitination of PCNA. Taken together, these results suggest that HERC2 and RNF8 promote TLS past abasic sites and UV-lesions at or very close to stalled replication forks.

1. Introduction

Replication involves a complex and fragile enzymatic reaction that can readily be disrupted by template DNA damage. To restart arrested replication, cells have evolved two post-replicational repair (PRR) pathways, translesion synthesis (TLS) and template switching by homologous recombination (HR). HR facilitates transient switching of replication from the damaged template strand to the newly synthesized sister chromatid [1–6]. In addition to template switching by HR, replication blockage is released by employing specialized TLS polymerases such as DNA polymerases η and ζ (Polη and Polζ) [7–9]. The deployment of the Y-family TLS polymerases is controlled by PCNA ubiquitination at K164 and by the non-catalytic function of the Y-family polymerase REV1 [10–13]. RAD18 ubiquitin ligase is responsible for the mono-ubiquitination in *Saccharomyces cerevisiae*, the chicken DT40 B lymphocyte cell line and mammalian cells [10–14].

HERC2 is a HECT domain E3 ubiquitin ligase and one of the largest genes in the vertebrate genome. It has been shown to play a role in control of nucleotide excision repair by ubiquitinating and degrading XPA [15,16]. It also plays a role in double strand break repair by facilitating the assembly of the Ubc13 ubiquitin-conjugating enzyme with the ubiquitin ligase RNF8 [17,18]. HERC2 also associates with RNF168, another ubiquitin ligase operating downstream of RNF8 in DSB repair [17,19–21]. RNF168 amplifies the RNF8-dependent histone ubiquitination by targeting H2A-type histones and by promoting the formation of lysine 63-linked ubiquitin conjugates [19,20]. These modifications

Abbreviations: AID, activation-induced deaminase; DSB, double strand break; HR, homologous recombination; Ig V gene, immunoglobulin variable gene; MMS, methyl methanesulfonate; PCNA, proliferating cell nuclear antigen; XPA, xeroderma pigmentosum complementation group A; PRR, post-replication repair; TLS, translesion DNA synthesis.

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http://dx.doi.org/10.1016/j.dnarep.2016.02.002
1568-7864/

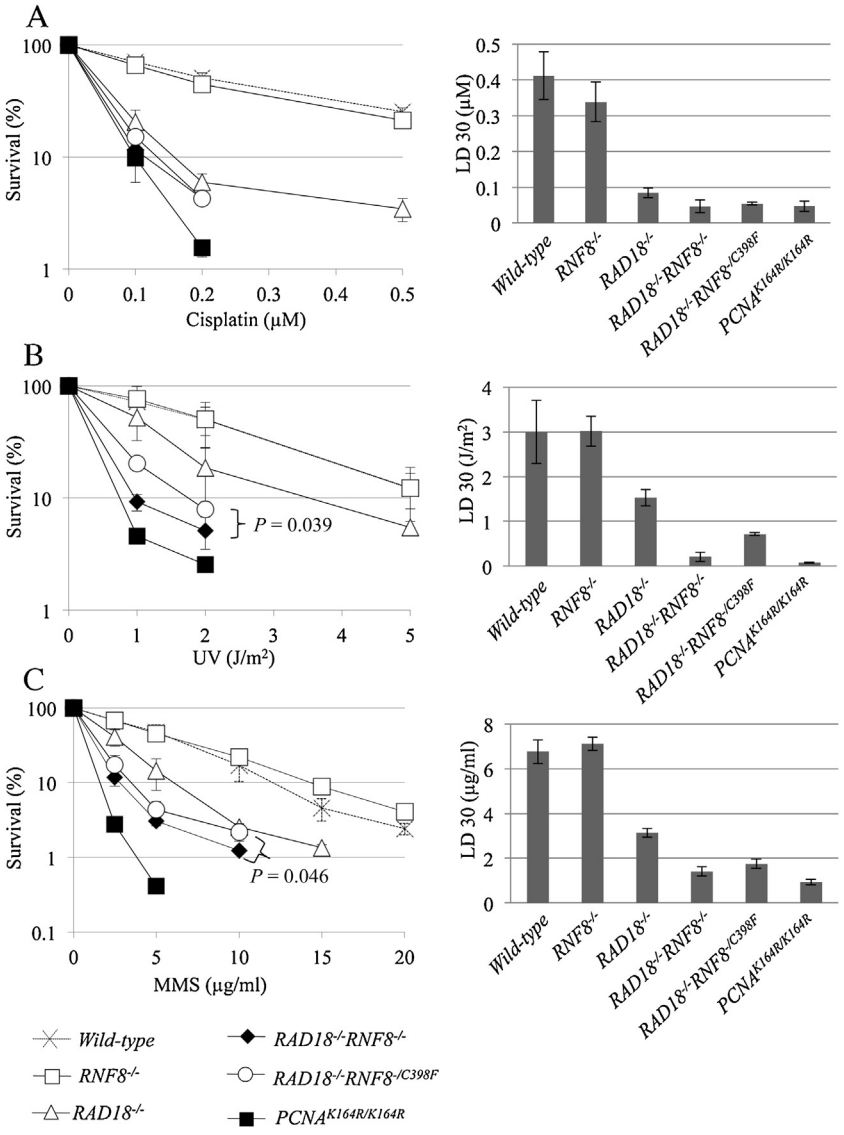

Fig. 1. Cellular sensitivity to DNA-damaging agents. (A–C) Cellular sensitivity to cisplatin (A), UV (B), and MMS (C) were analyzed. Survival rate was calculated as the percentage of surviving cells treated with DNA-damaging agents relative to the untreated surviving cells. The concentration or dose is displayed on the *x*-axis on a linear scale, while the survival rate is displayed on the *y*-axis on a logarithmic scale. *P*-values were calculated by Student's *t*-test. Lethal dose 30% (LD30) is the concentration of DNA damaging agents that reduces cellular survival to 30% relative to cells non-treated with DNA damaging agents. LD30 was calculated by the statistics software, R. Error bars show the standard deviation of the mean of at least three independent experiments.

orchestrate the accumulation of 53BP1 and BRCA1 to DNA lesions [19,20]. A recently published study showed that RNF8 but not RNF168 is responsible for the K63-linked ubiquitination of H1-type linker histones [22]. While the contribution of HERC2 to the ubiquitination response to DNA double strand breaks and HR-mediated DSB repair has been established, its role in restoring stalled replication forks has not been explored. Indeed, it remains unclear whether RNF8 is involved in DNA damage response other than DSB repair.

The chicken DT40 B lymphocyte cell line provides a unique opportunity to specifically analyze the involvement of DNA damage repair proteins in PRR pathways (Fig. S1) through examination of the immunoglobulin variable (Ig V) gene diversification during *in vitro* culture [23]. This diversification is driven by release of abasic site-mediated replication blocks either by TLS, which results in non-templated point mutations, or by HR, which drives gene conversion with a set of homeologous pseudogenes [9,24]. These diversification processes are initiated by deamination of deoxycytidine by activation-induced deaminase (AID) to generate uracil [25,26], which is subsequently excised, leaving an abasic site [27–29]. TLS releases replication forks stalling at abasic sites, leading to Ig V non-templated mutations at G/C pairs (Ig hypermutation) [30–34]. Both gene conversion tracts and the spectrum of non-templated point mutations can be evaluated by identifying Ig V nucleotide sequence variations during clonal expansion of DT40 cells.

In this study, we show that *HERC2*$^{-/-}$, *RNF8*$^{-/-}$ and *HERC2*$^{-/-}$/*RNF8*$^{-/-}$ cells all exhibit a several-fold decrease in the rate of Ig hypermutation. The *HERC2*$^{-/-}$ and *RNF8*$^{-/-}$ mutants are also defective in the maintenance of replication fork progression immediately after exposure of cells to UV. These data are consistent with HERC2 and RNF8 promoting TLS past abasic sites at or very close to stalled replication forks.

2. Materials and methods

2.1. Cell culture

RAD18$^{-/-}$, *RAD18*$^{-/-}$/*RNF8*$^{-/-}$, *RNF8*$^{-/-}$, *RNF8*$^{-/C398F}$, *RAD18*$^{-/-}$/*RNF8*$^{-/C398F}$ DT40 cells [21,35] and *HERC2*$^{-/-}$, *HERC2*$^{-/-}$/*RNF8*$^{-/-}$ DT40 cells [21] were previously generated.