Available online at www.sciencedirect.com
SCIENCE DIRECT®
DNA Repair 4 (2005) 649–654
DNA REPAIR
www.elsevier.com/locate/dnarepair

Reduced repair of DNA double-strand breaks by homologous recombination in a DNA ligase I-deficient human cell line

Julie Della-Maria Goetz [a], Teresa A. Motycka [a], Minguang Han [b], Maria Jasin [b], Alan E. Tomkinson [a,*]

[a] *Radiation Oncology Research Laboratory, Department of Radiation Oncology and Greenebaum Cancer Center, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201, USA*
[b] *Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*

Received 1 December 2004; accepted 10 February 2005
Available online 7 April 2005

Abstract

Genetic and biochemical studies of mammalian DNA ligase I indicate that this multifunctional enzyme plays a key role in the completion of DNA replication and certain DNA excision repair pathways. However, the involvement of DNA ligase I in DNA double-strand break repair has not been examined. Here we have determined the effect of DNA ligase I-deficiency on the frequency of homologous recombination initiated by a site-specific DNA double-strand break. We found that expression of wild-type DNA ligase I in a human DNA ligase I mutant cell line significantly increased the frequency of homologous recombination. Notably, the ability of DNA ligase I to promote the recombinational repair of DNA double-strand breaks was dependent upon its interaction with proliferating cell nuclear antigen. Thus, our results demonstrate that DNA ligase I-deficiency reduces recombinational repair of DNA double-strand breaks.

Keywords: DNA ligase I; DNA double-strand break repair; Homologous recombination; PCNA

1. Introduction

DNA double-strand breaks (DSBs) are a particularly dangerous lesion because there is no undamaged strand in the duplex to act as the template to guide repair. Although eukaryotes possess several mechanistically distinct pathways to repair DSBs, these repair pathways share a common last step, DNA ligation. In the yeast *Saccharomyces cerevisiae* there are two genes encoding DNA ligases, *CDC9* and *DNL4* [1,2]. Based on genetic analysis, it appears that the product of the *DNL4* gene functions only in the repair of DSBs by non-homologous end-joining (NHEJ) [2,3], a pathway in which broken DNA ends are simply brought together, processed and then joined. In contrast, the *CDC9* gene product is multifunctional, participating in DNA replication and DNA excision repair [1,4,5]. Moreover, it appears likely that Cdc9 also participates in homology-dependent recombinational repair [4]. Analysis of the role of Cdc9 in homologous recombination is difficult for a couple of reasons. Firstly, *CDC9* is an essential gene. Secondly, reduced levels of Cdc9 DNA ligase activity give rise to increased strand breaks, such as unligated Okazaki fragments and incomplete DNA repair events, that in turn stimulate recombination [4,5].

In mammals, the *LIG1* and *LIG4* genes are homologs of the yeast *CDC9* and *DNL4* genes, respectively [1,2]. Similar to the homologous yeast enzymes, mammalian DNA ligase I participates in DNA replication [6–9] and excision repair [10] whereas DNA ligase IV completes DSB repair by the NHEJ pathway [3,11]. However, mammals also possess an additional *LIG* gene, *LIG3*, that encodes three distinct gene products involved in nuclear DNA repair, mitochondrial DNA metabolism and germ cell differentiation. There is accumulating evidence that, in mammals, the products of the *LIG3* gene have taken over from or can partially substitute for functions carried out by Cdc9 DNA ligase in yeast. For example,

* Corresponding author. Tel.: +1 410 706 2365; fax: +1 410 706 3000.
E-mail address: atomkinson@som.umaryland.edu (A.E. Tomkinson).

1568-7864/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2005.02.004

DNA ligase: IIα completes the short patch subpathway of base excision repair (BER) in mammals [12] whereas yeast BER is completed by Cdc9 DNA ligase [5]. In addition, the viability of mouse *ligI* null cells suggests that one of the other DNA ligases, presumably DNA ligase LIIα, may be able to substitute, at least partially, for DNA ligase I in DNA replication [13].

At the present time, very little is known about the roles of the *LIG1* and *LIG3* gene products in homology-dependent recombinational repair. Interestingly, mutant mammalian cells with reduced DNA ligase III activity have dramatically elevated levels of spontaneous sister chromatid exchanges (SCE)s [14]. By contrast, a human DNA ligase I-deficient cell line has a defect in the joining of Okazaki fragments but only slightly elevated levels of spontaneous SCE [15]. The human DNA ligase I-deficient cell line 46BR.1G1 was established from an immunodeficient individual with growth and developmental abnormalities [10,16]. In this cell line, the mutated *ligI* allele encodes a version of DNA ligase I with about 10-fold less activity than the wild-type enzyme [17]. As noted above, these cells have a defect in Okazaki fragment joining but near normal levels of spontaneous SCE. Notably, the reiteration of the mutation found in the 46BR.1G1 *ligI* allele in a mouse model resulted in an increased incidence of cancer [18]. In this study, we have integrated an SCneo reporter construct containing a single I-*Sce*I site [19] into the genome of 46BR.1G1 cells and used this cell system to examine the role of DNA ligase I in the repair of a site-specific DSB.

2. Materials and methods

2.1. Establishment of derivatives 46BR.1G1 with a single integrated copy of the SCneo reporter construct

46BR.1G1 cells were transfected with the SCneo plasmid (Fig. 1A [19]). Colonies were selected in culture medium (Dulbecco's Modified Eagle Medium (DMEM), 10% fetal

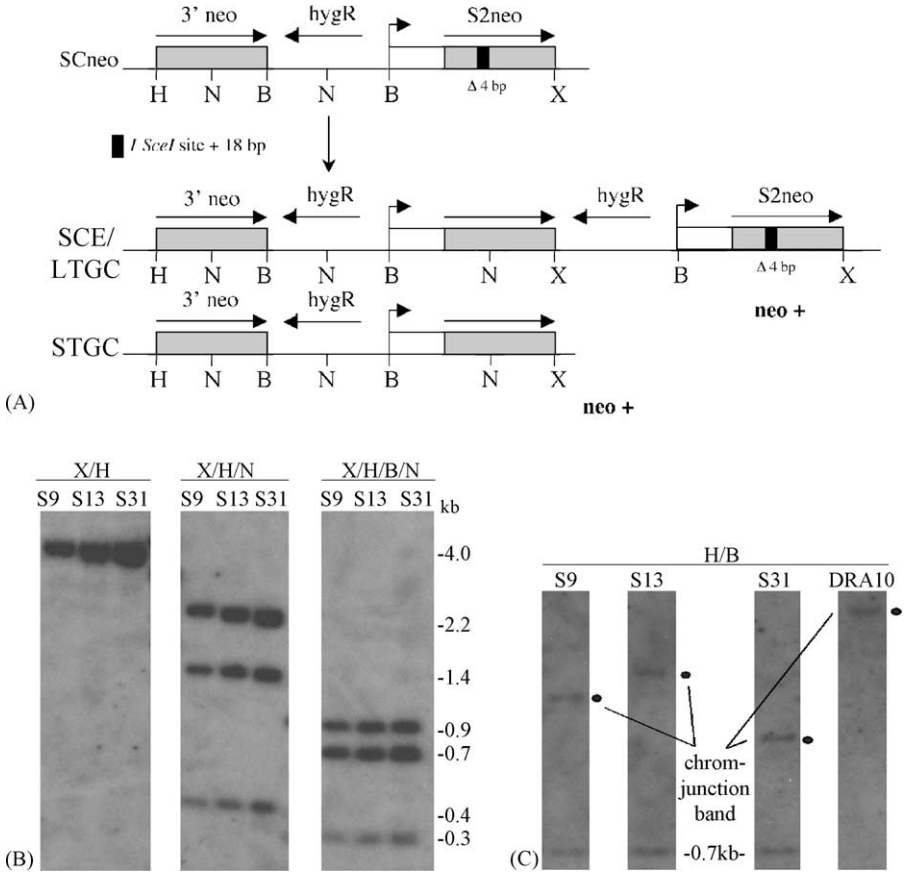

Fig. 1. Generation of derivatives of 46BR.1G1 containing an integrated copy of the SCneo reporter construct. (A) Diagram of the SCneo reporter construct and the structure of neo+ versions of SCneo generated by I-*Sce*I induced homology-dependent repair pathways. SCneo consists of a 3′ fragment of the *neo* gene upstream of the S2neo gene. S2neo is mutated at an *Ncol* site by deletion of 4 bp (Δ 4 bp) of neo coding sequence and insertion of the 18 bp *I-Scel* site. Repair of the DSB by either sister chromatid exchange (SCE) or long tract gene conversion (LTGC) and by short tract gene conversion (STGC) generates a functional *neo* gene (neo+) with the indicated structures, neo, neomycin; hygR, hygromycin. (B) Southern blotting of genomic DNA from the 46BR.1G1 derivatives S9, S13 and S31. DNA was digested with the indicated enzymes and then probed with SCneo to demonstrate that the integrated construct is intact. (C) Southern blotting of genomic DNA from the 46BR.1G1 derivatives S9, S13 and S31 and DRA10, a control cell line derived from DRneo [23]. DNA was digested with the indicated enzymes and then probed with SCneo. The presence of only one junction band (chrom. junction band) indicates single copy integration. *Bam*III, B; *Hin*dIII, H; N, *Nco*I; X, *Xho*I.