Accepted Manuscript

Title: TDP1 is required for efficient non-homologous end joining in human cells

Authors: Jing Li, Matthew Summerlin, Karin C. Nitiss, John L. Nitiss, Leslyn A. Hanakahi

PII: S1568-7864(17)30096-4
DOI: https://doi.org/10.1016/j.dnarep.2017.10.003
Reference: DNAREP 2431

To appear in: DNA Repair

Received date: 29-3-2017
Revised date: 9-10-2017
Accepted date: 10-10-2017

Please cite this article as: Jing Li, Matthew Summerlin, Karin C. Nitiss, John L. Nitiss, Leslyn A. Hanakahi, TDP1 is required for efficient non-homologous end joining in human cells, DNA Repair https://doi.org/10.1016/j.dnarep.2017.10.003

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Title: TDP1 is required for efficient non-homologous end joining in human cells.

Authors: Jing Li¹, Matthew Summerlin¹, Karin C. Nitiss², John L Nitiss², Leslyn A Hanakahi²*

Department of ¹Medicinal Chemistry and Pharmacognosy, and of ²Biopharmaceutical Sciences, College of Pharmacy, University of Illinois, Chicago. 1601 Parkview Ave. Rockford, IL, 61107.

*to whom correspondence should be addressed. Hanakahi@uic.edu, (815) 395-5924

Highlights
• Loss of TDP1 significantly reduces end joining of restriction enzyme induced DSBs in human cells
• TDP1-deficient human cells also show reduced end-joining fidelity, with fewer perfect junctions and an increased fraction of insertion events.
• A phosphomimetic mutation in the N-terminus of TDP1 also results in lower levels of NHEJ, suggesting that phosphorylation of TDP1 regulate its participation in NHEJ

ABSTRACT

Tyrosyl-DNA phosphodiesterase 1 (TDP1) can remove a wide variety of 3' and 5' terminal DNA adducts. Genetic studies in yeast identified TDP1 as a regulator of non-homologous end joining (NHEJ) fidelity in the repair of double-strand breaks (DSBs) lacking terminal adducts. In this communication, we show that TDP1 plays an important role in joining cohesive DSBs in human cells. To investigate the role of TDP1 in NHEJ in live human cells we used CRISPR/cas9 to produce TDP1-knockout (TDP1-KO) HEK-293 cells. As expected, human TDP1-KO cells were highly sensitive to topoisomerase poisons and ionizing radiation. Using a chromosomally-integrated NHEJ reporter substrate to compare end joining between wild type and TDP1-KO cells, we found that TDP1-KO cells have a 5-fold reduced ability to repair I-SceI-generated DSBs. Extracts prepared from TDP1-KO cells had reduced NHEJ activity in vitro, as compared to extracts from wild type cells. Analysis of end-joining junctions showed that TDP1 deficiency reduced end-joining fidelity, with a significant increase in insertion events, similar to previous observations in yeast. It has been reported that phosphorylation of TDP1 serine 81 (TDP1-S81) by ATM and DNA-PK stabilizes TDP1 and recruits TDP1 to sites of DNA damage. We found that end joining in TDP1-KO cells was partially restored by the non-phosphorylatable mutant TDP1-S81A, but not by the phosphomimetic TDP1-S81E. We previously reported that TDP1 physically interacted with XLF. In this study, we found that XLF binding by TDP1 was reduced 2-fold by the S81A mutation, and 10-fold by the S81E phosphomimetic mutation. Our results demonstrate a novel role for TDP1 in NHEJ in human cells. We hypothesize that TDP1 participation in human NHEJ is mediated by interaction with XLF, and that TDP1-XLF interactions and subsequent NHEJ events are regulated by phosphorylation of TDP1-S81.

Keywords: Tyrosyl-DNA phosphodiesterase 1, TDP1, non-homologous end joining, NHEJ, and XLF

Abbreviations: Tyrosyl-DNA phosphodiesterase 1, TDP1. TDP1-knockout, TDP1-KO. non-homologous end joining, NHEJ.

1. Introduction

Tyrosyl-DNA phosphodiesterase 1 (TDP1) was first identified in yeast as a factor that removes trapped
دریافت فوری
متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات