DNA REPAIR 6 (2007) 781–788

available at www.sciencedirect.com

ScienceDirect

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

Non-homologous end-joining for repairing I-SceI-induced DNA double strand breaks in human cells

*Masamitsu Honma**, *Mayumi Sakuraba, Tomoko Koizumi,*
Yoshio Takashima, Hiroko Sakamoto, Makoto Hayashi

Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

ARTICLE INFO

Article history:
Received 12 July 2006
Received in revised form
4 December 2006
Accepted 4 January 2007
Published on line 12 February 2007

Keywords:
DNA double strand break (DSB)
Non-homologous end-joining (NHEJ)
Homologous recombination (HR)
I-SceI
Deletion
Genomic integrity

ABSTRACT

DNA double strand breaks (DSBs) are usually repaired through either non-homologous end-joining (NHEJ) or homologous recombination (HR). While HR is basically error-free repair, NHEJ is a mutagenic pathway that leads to deletion. NHEJ must be precisely regulated to maintain genomic integrity. To clarify the role of NHEJ, we investigated the genetic consequences of NHEJ repair of DSBs in human cells. Human lymphoblastoid cell lines TSCE5 and TSCE105 have, respectively, single and double I-*Sce*I endonuclease sites in the endogenous thymidine kinase gene (*TK*) located on chromosome 17q. I-SceI expression generated DSBs at the *TK* gene. We used the novel transfection system (Amaxa Nucleofector) to introduce an I-SceI expression vector into the cells and randomly isolated clones. We found mutations involved in the DSBs in the *TK* gene in 3% of TSCE5 cells and 30% of TSCE105 cell clones. Most of the mutations in TSCE5 were small (1–30 bp) deletions with a 0–4 bp microhomology at the junction. The others consisted of large (>60) bp deletions, an insertion, and a rearrangement. Mutants resulting from interallelic HR also occurred, but infrequently. Most of the mutations in TSCE105, on the other hand, were deletions that encompassed the two I-*Sce*I sites generated by NHEJ at DSBs. The sequence joint was similar to that found in TSCE5 mutants. Interestingly, some mutants formed a new I-*Sce*I site by perfectly joining the two original I-*Sce*I sites without deletion of the broken-ends. These results support the idea that NHEJ for repairing I-SceI-induced DSBs mainly results in small or no deletions. Thus, NHEJ must help maintain genomic integrity in mammalian cells by repairing DSBs as well as by preventing many deleterious alterations.

1. Introduction

DNA double strand breaks (DSBs) are the most dangerous form of DNA damage. They can be caused by ionizing radiation (IR) or radiometric chemicals, and they can occur spontaneously during DNA replication. Other DNA damage, such as single strand breaks, easily convert to DSBs when a replication fork encounters them [1,2]. The non- or misrepair of DSBs can cause cell death or neoplastic transformation [3,4], so the accurate repair of DSBs is important for maintaining genomic integrity [5]. DSBs are generally repaired through non-homologous end-joining (NHEJ) or homologous recombination (HR) [6,7]. NHEJ joins sequences at the broken ends, which have little or no homology, in a non-conservative manner, and some genetic information is lost. HR, on the other hand, requires extensive tracts of sequence homology and is

---

* *Corresponding author*. Tel.: +81 3 3700 9847; fax: +81 3 3700 2348.
E-mail address: honma@nihs.go.jp (M. Honma).

1568-7864/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2007.01.004

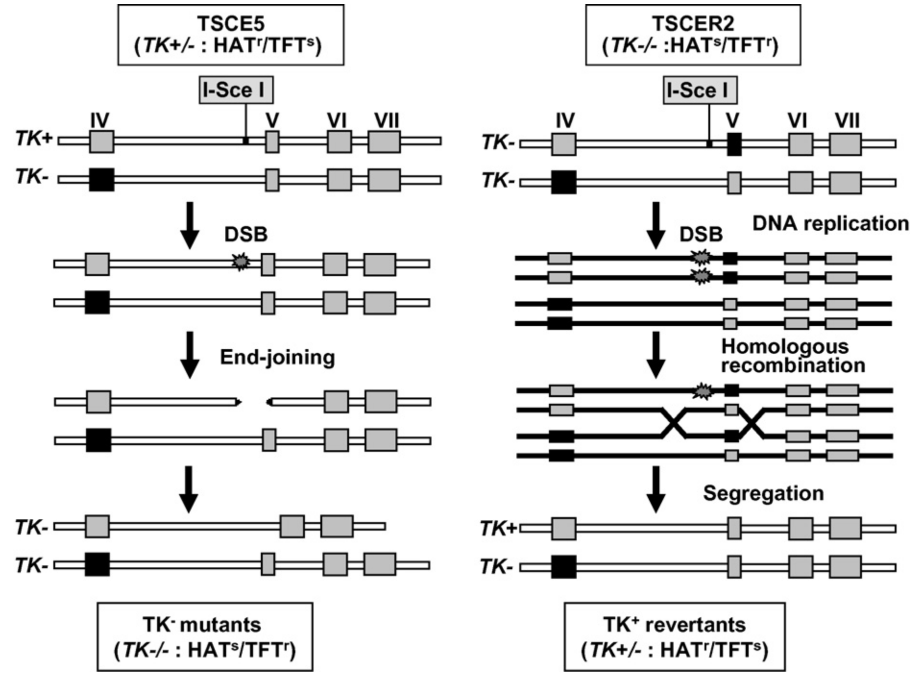

Fig. 1 – Schematic representation of the experimental system. Shadowed and closed rectangles represent the wild type and mutant exons of the *TK* gene, respectively. In TSCE5 cells, when a DSB at the I-*Sce*I site is repaired by NHEJ and causes an exon 5 deletion, TK-deficient mutants are selected in TFT medium. In TSCER2 cells, when a DSB at the I-*Sce*I site is repaired by HR, TK-proficient revertants are selected in HAT medium.

basically error-free [8]. HR is the primary DSB repair pathway in yeast and prokaryotes, but NHEJ is believed to be the primary pathway in mammalian cells [9]. HR is preferable to NHEJ because it is error-free, but NHEJ may have a different way to maintain genomic integrity.

We previously developed a human cell system to trace the fate of a DSB occurring in an endogenous single copy gene (Fig. 1) [10]. The human lymphoblastoid cell line, TSCE5, is heterozygous (+/−) and TSCER2 is compound heterozygous (−/−) for the thymidine kinase gene (*TK*), and both have an I-*Sce*I endonuclease site in intron 4. DSBs can be generated at the I-*Sce*I site by the introduction of an I-SceI enzyme expression vector. When DSBs occur at the *TK* locus, NHEJ in TSCE5 cells produces TK-deficient mutants, while HR between the alleles produces TK-proficient revertants in TSCER2 cells. Positive–negative drug selection for the TK phenotypes permits the distinction between NHEJ and HR repair mechanisms. Using the same system, we previously found that almost all I-SceI-induced DSBs in human cells are repaired by NHEJ and result in mainly 100–4000 bp deletions [10]. Drug selection, however, does not recover cells with genetic changes that are too small to influence TK function, and the resulting spectrum of mutations and reversions may be biased quantitatively as well as qualitatively.

To better understand the fate of DSBs in human cells, we randomly isolated non-selected clones after introducing DSBs and directly analyzed their DNA. A novel transfection system (Amaxa Nucleofector^{TM}) can introduce the I-SceI expression vector into most of cell population [11] and efficiently produces DSBs at the *TK* gene. With this improved method, we were able to detect cells with deletions at DSBs without drug selection and to trace the fate of DSBs without bias. We also developed a new cell line that has two I-*Sce*I sites in the *TK* gene and can be used as a model for clustering DSBs. DNA sequence analysis of the mutants in this strain revealed that both single and double DSBs were repaired predominantly by NHEJ, producing only small genetic changes, or none. We discuss how NHEJ maintains genomic integrity.

2. Materials and methods

2.1. *Human cell lines for detecting NHEJ and HR induced by a single DSB*

Human lymphoblastoid cell lines TSCE5 and TSCER2 were previously created from TK6 cells [10], which are heterozygous for a point mutation in exon 4 of the *TK* gene (*TK*+/−) (Fig. 1). TSCE5 has a 31bp DNA flagment containing the 18 bp I-*Sce*I site inserted 75bp upstream of exon 4 of the *TK*+ allele and retains TK function. TSCER2 is a TK-deficient mutant spontaneously arising from TSCE5. It has a point mutation (G:A transition) at 23bp of exon 5 of the *TK*+ allele of TSCE5. TSCER2 is compound heterozygote (*TK*−/−) for the *TK* gene. NHEJ for a DSB occurring at the I-*Sce*I site results in TK-deficient mutants in TSCE5 cells, while HR between the alleles produces TK-proficient revertants in TSCER2 cells.

2.2. *I-SceI expression and isolation of mutant clones*

We introduced the I-SceI expression vector (pCBASce) by suspending 5×10^6 cells in 0.1 ml Nucleofector solution V (Amaxa