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Brief report

Comparison of nonhomologous end joining and homologous recombination in human cells

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ABSTRACT

The two major pathways for repair of DNA double-strand breaks (DSBs) are homologous recombination (HR) and nonhomologous end joining (NHEJ). HR leads to accurate repair, while NHEJ is intrinsically mutagenic. To understand human somatic mutation it is essential to know the relationship between these pathways in human cells. Here we provide a comparison of the kinetics and relative contributions of HR and NHEJ in normal human cells. We used chromosomally integrated fluorescent reporter substrates for real-time *in vivo* monitoring of the NHEJ and HR. By examining multiple integrated clones we show that the efficiency of NHEJ and HR is strongly influenced by chromosomal location. Furthermore, we show that NHEJ of compatible ends (NHEJ-C) and NHEJ of incompatible ends (NHEJ-I) are fast processes, which can be completed in approximately 30 min, while HR is much slower and takes 7 h or longer to complete. In actively cycling cells NHEJ-C is twice as efficient as NHEJ-I, and NHEJ-I is three times more efficient than HR. Our results suggest that NHEJ is a faster and more efficient DSB repair pathway than HR.

1. Introduction

Double-stranded DNA break (DSB) is a dangerous DNA lesion. If left unrepaired DSBs result in massive loss of genetic information, chromosomal aberrations, or cell death. The two major pathways for the repair of DSBs, which differ in the fidelity and template requirements, are nonhomologous end joining (NHEJ) and homologous recombination (HR) [1]. NHEJ modifies the broken DNA ends, and ligates them together with no regard for homology, generating deletions or insertions [2]. In contrast, HR uses an undamaged DNA template to repair the break, leading to the reconstitution of the original sequence [3].

Both DSB repair pathways play important roles in mammalian DSB repair [4,5]. The exact mechanism by which NHEJ and HR interact, and how the choice is made between the two pathways remains unclear. NHEJ and HR may compete for a DSB, or the choice may be determined by the structure of the broken ends [6]. To understand human somatic mutation it is essential to know the relationship between these pathways in human cells. Surprisingly, the efficiency and kinetics of HR and NHEJ have not been directly compared.

Here we employed sensitive fluorescent reporter assays to examine NHEJ and HR in human cells. Reporter cassettes were chromosomally integrated in normal human fibroblasts immortalized by ectopic expression of telomerase, which retain all characteristics of untransformed primary cells [7]. The assays are based on the reconstitution of a functional GFP gene, where the completion of NHEJ or HR is monitored in real-time by the appearance of green cells. Using these assays

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we compared the kinetics of NHEJ and HR, and determined the ratio between the two processes in multiple genomic locations. We show that NHEJ is a faster and more efficient pathway than HR in human cells.

2. Materials and methods

2.1. Cell culture

Cells were cultured at 37 °C in a 5% CO_2, 3% O_2 incubator, in EMEM media supplemented with 15% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin.

2.2. Construction of cell lines for detecting NHEJ and HR efficiency

To generate reporter cell lines HCA2-hTERT cells were transfected with 0.5 μg of linearized NHEJ-I, NHEJ-C, or HR reporter constructs. G418, at 1 mg/ml, was added to the media 1 day post-transfection. Colonies were picked after 8–10 days on selection. Genomic DNA was then extracted and analyzed by Southern blotting with 3′ and 5′ probes to confirm that the cell lines contained a single integrated copy of the reporter cassettes. The numbers of cell lines analyzed with Southern blotting were 47 for NHEJ-I, 38 for NHEJ-C, and 28 for HR. Of those cell lines, 14 NHEJ-I cell lines, 14 NHEJ-S cell lines, and 7 HR cell lines had single reporter cassettes. Seven cell lines of each type were randomly picked for further research.

2.3. Transfections

The transfections were performed using Amaxa Nucleofector; program U-20. In each transfection 2 million cells were transfected.

2.4. FACS analysis

For analysis of NHEJ and HR cells were harvested, resuspended in ∼1 ml 1× PBS, put on ice, and run on a FACS machine. GFP and DsRed fluorescence was analyzed using the red-versus-green plot as described previously [8]. FACS analysis was performed on a FACS Calibur instrument, and data were analyzed using CellQuest software.

2.5. Antibodies

I-SceI expression was analyzed with anti-HA 2367 antibody (Cell Signaling). Actin expression was detected with sc-8432 antibody (Santa Cruz).

3. Results

3.1. Reporter cell lines for analysis of NHEJ and HR

To investigate the roles of NHEJ and HR in DSB repair in normal human cells we generated a series of reporter cell lines containing chromosomally integrated GFP-based reporter constructs. The reporter cassette for detecting NHEJ [8] contains a GFP gene with an artificially engineered 3 kb intron from the Pem1 gene (GFP-Pem1). The Pem1 intron contains an adenoviral exon flanked by recognition sequences for I-SceI endonuclease in direct (Fig. 1a) or inverted (Fig. 1b) orientation, which generate compatible (Fig. 1d) or incompatible ends (Fig. 1e) respectively. Incompatible DNA ends best mimic the naturally occurring DSBs. An un-rearranged NHEJ cassette is GFP negative since the adenoviral exon disrupts the GFP ORF. Upon induction of DSBs by the expression of I-SceI, the adenoviral exon is removed and NHEJ restores function of the GFP gene. This reporter can detect a wide spectrum of NHEJ events since the intron can tolerate deletions and insertions. The NHEJ reporters will not detect the events where only one I-SceI site is digested and then repaired without a deletion. Therefore, NHEJ frequencies measured by these constructs may be an underestimate.

The HR reporter (Fig. 1c) is built on the same GFP-Pem1 basis as the NHEJ reporter. In the HR reporter, the first exon of GFP-Pem1 contains a 22 bp deletion combined with the insertion of three restriction sites, I-SceI-HindIII-I-SceI, which are used for inducing DSBs. The deletion ensures that GFP cannot be reconstituted by an NHEJ event. The two I-SceI sites are in an inverted orientation, so that I-SceI digestion leaves incompatible ends (Fig. 1c). The first copy of GFP-Pem1 is followed by a promoter-less/ATG-less first exon and intron of GFP-Pem1. The intact construct is GFP-negative. Upon induction of a DSB by I-SceI digestion the functional GFP gene will be reconstituted by intramolecular or intermolecular gene conversion between the two mutated copies of the first GFP-Pem1 exon. Since the second copy of the GFP gene is lacking the first ATG codon and the second exon, crossing over or single-strand annealing will not restore the GFP activity. This design allows for the exclusive detection of gene conversion, which is the predominant HR pathway in mammalian cells [9].

Since the sequences surrounding the I-SceI sites differ between the NHEJ and HR constructs there is a possibility that the cleavage of the HR substrate occurs more slowly or less efficiently. To test this we digested the plasmids containing the NHEJ and HR substrates with I-SceI enzyme *in vitro*. We did not detect any difference in the efficiency of digestion (data not shown). This experiment does not completely rule out that a difference exists *in vivo*, but makes it highly unlikely.

The efficiency of DSB repair may be affected by chromosomal position. Therefore, to obtain a representative picture of the roles of NHEJ and HR in DSB repair we generated multiple randomly integrated reporter cell lines with each construct. The NHEJ or HR reporter cassettes were stably integrated on a chromosome of HCA2 normal human fibroblasts immortalized by expression of hTERT (HCA2-hTERT). Clones containing single un-rearranged copies of each reporter cassette were identified by Southern blot (data not shown), and seven independent cell lines of each kind were randomly chosen for further analysis. The cell lines were designated as follows. Cell lines containing a NHEJ cassette to measure NHEJ of *compatible* I-SceI ends (NHEJ-C) were designated S35c, S28c, S30c, S44c, S15c, S46c, S13a. The cell lines containing the cassette to measure NHEJ of *incompatible* I-SceI ends (NHEJ-I) were designated I26c, I29c, I13b, If16a, I9a, I7c, I4a. Finally, the cell lines containing HR construct were named H32c, H29c, H33c, H23c, H15c, H4b, H9b. Construction of the I9a and H15c cell lines was described previously, and formation of the expected NHEJ