Repeated sequences with strong tetraplex-forming potential generate double-strand breaks in yeast and high frequency chromosomal rearrangements in human sperm

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Motivation

Double-strand breaks (DSBs) are among the most cytotoxic forms of DNA damage and can lead to chromosomal aberrations, disruption of genomic integrity, and cancer (McKinnon and Caldecott, 2007; Shrivastav et al, 2008). Recently, a major role for non-B DNA conformations (such as cruciforms, left-handed Z-DNA, triplex DNA and tetraplex) in generating DSBs and genomic rearrangements has been revealed (Bacolla et al, 2004). While in S. cerevisiae long palindromic sequences (Nag and Kurst, 1997) and CAG repeat tracts (Jankowski et al, 2000) have been shown to form DSB during meiosis, no yeast data were available for tetraplex-forming sequences. We examined whether a repeated sequence in the SHANK3 gene, SHANK3-REP, which is the site of a recurrent breakpoint associated with chromosome 22q13 deletion syndrome and has a strong tetraplex forming potential, is able to induce DSBs in yeast and in human DNA.

Methods

We analyzed whether the SHANK3-REP is able to generate DSBs during meiosis in yeast. We also examined the frequency of SHANK3-REP-related deletions repaired by telomerase in human male germ cells and blood. We implemented a previously described scoring method (Burge et al, 2006; Todd 2007; Kikin et al, 2006) in the R environment (R Development Core Team, 2007) and retrieved all potential quadruplex-forming sequences together with their scores. We divided all the chromosomes into non-overlapping windows and assigned to each element of the partition the sum of the scores of all putative quadruplex sequences contained in each window. We recorded the chromosomal position of the 46 windows with the highest quadruplex scores (equally distributed on both strands and on the p-arm and q-arm) according to the following additional criteria: they should be located within 4 Mb from a telomere; the repeat should not be longer than 1.5 kb; the repeat should not be contained in a larger CpG island. In addition, we selected by BLAT analysis (http://genome.ucsc.edu/cgi-bin/hgBlat) the three sequences most similar to SHANK3-REP. For each sequence, we set up and performed a specific ACP-PCR for telomere assay on 100 ng aliquotsof 20 sperm samples and 11 blood samples from normal subjects. Amplified fragments were cloned and sequenced.

Results

We show that the SHANK3-REP sequence generates DSBs during meiosis in yeast. We have also analyzed the incidence of deletion-specific PCR products in sperm and blood DNA from normal individuals, and report high incidence of deletions healed by the addition of telomeric sequences in sperm DNA, comparable to the incidence of de novo constitutional t(11;22) translocations and Î±-globin gene deletions, and lower but detectable incidence in blood. We have mapped tetraplex-forming sequences in the human genome and shown their association with several genomic features, including meiotic recombination hotspots. We have also demonstrated the occurrence of high frequency terminal deletions in additional repeats with strong tetraplex-forming potential. Our data indicate a major role for quadruplex-forming sequences in human chromosomal rearrangements.

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