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Relationships between replication timing and GC content of cancer-related genes on human chromosomes 11q and 21q

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ABSTRACT

The human genome is composed of large-scale compartmentalized structures, including long G+C% (GC%) mosaic structures and replication-timing zones, which are related to chromosome band zones. Previously, we measured replication timing along the entire lengths of human chromosomes 11q and 21q at the sequence level, and it was suggested that the transition regions of replication timing from early to late S-phase coincided with "unstable" regions of the genome associated with increased DNA damage. In the present study, we measured replication timing of 15 known oncogenes and tumor suppressor genes on human chromosomes 11q and 21q using two human cell lines (THP-1 and Jurkat). We found unusual relationships between replication timing and the GC content of the genomic regions in which these cancer-related genes were located. Many of these genes showed similar replication timing between the two cell lines, and the majority replicated intermediately between early and late in both cell lines. On the other hand, more than half of these genes were located at very GC-rich (50–55 GC%) regions. In addition, we analyzed the exact relationships between early/late-switch regions of replication timing where cancer-related genes were located, and GC% transitions in and around five R/G-chromosomal band boundaries (each ca. 4 Mb) by using newly designed PCR primer sets. We found that the majority of cancer-related genes including oncogenes were located in GC-rich isochores close to GC% transitions within early/late-switch regions of replication timing, many of which replicated intermediately between the early and late S-phase. Unusual relationships between replication timing and GC content of the genomic regions in which cancer-related genes were located may be related to the molecular mechanisms of genomic instability associated with increased DNA damage.

1. Introduction

In warm-blooded vertebrates, including humans, the genome is composed of large-scale compartmentalized structures, including long-range G+C% (GC%) mosaic structures called isochores (Bernardi et al., 1985) and replication-timing zones (Ikemura, 1985; Aota and Ikemura, 1986; Holmquist, 1989; Gardiner et al., 1990; Pilia et al., 1993; Saccone et al., 1999). The compartmentalized structures correlate with chromosome bands. Giemsa-dark G-band zones, which replicate late in S phase, are composed mainly of AT-rich sequences; T bands (a subgroup of Giemsa-pale R bands) replicate very early and are composed of GC-rich sequences. Ordinary R bands replicate early and appear to have a heterogeneous GC content (Ikemura and Aota, 1988; Bernardi, 1989; Ikemura et al., 1990; Ikemura and Wada, 1991; Bernardi, 1993; Craig and Bickmore, 1993; Saccone et al., 1993). Therefore, in regions containing R/G-chromosomal band boundaries, it is expected that replication timing switches from early to late S-phase, and that DNA sequences change from GC-rich to AT-rich.

Previously, we measured replication timing along the entire lengths of human chromosomes 11q and 21q at the sequence level and found that early zones were more GC-rich and gene-rich than late zones. In the transition regions of replication timing from early to late, concentrated occurrence of cancer-related genes including oncogenes was observed and SNP frequency was high (Watanabe et al., 2002). In addition, we found that amplicons of human chromosome 11q, which included cancer-related genes, were located in the early/late-switch regions of replication timing (Watanabe et al., 2004). Therefore, it was suggested that the early/late-switch regions of replication timing coincided with "unstable" regions of the genome associated with increased DNA damage.

Many human genes including oncogenes and tumor suppressor genes have been mapped precisely in the genome. In the present study, we characterized replication timing and GC content of 15 known oncogenes and tumor suppressor genes on chromosomes 11q and 21q in two human cell lines. In addition, in these chromosomes we analyzed five human R/G-chromosomal band boundaries (each ca. 4 Mb) containing cancer-related genes, and clarified exact

Abbreviations: Mb, megabase; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; STS, sequence-tagged site.

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relationships between the early/late-switching of replication timing and GC% transition in and around these R/G-chromosomal band boundaries at the sequence level.

2. Materials and methods

2.1. Cell cycle fractionation and isolation of newly replicated DNA

THP-1 (2*n*=46, XY; a human acute monocytic leukemia cell line) and Jurkat (a human prototypical $CD28^+$ T cell leukemia cell line) were obtained from the Health Science Research Resources Bank (Tokyo, Japan) (Tsuchiya et al., 1980). The two human cell lines were labeled for 60 min with 75 μM 5′-bromo-2′-deoxyuridine (BrdU; Roche). The BrdU labeling, cell-cycle fractionation and isolation of newly replicated DNA were performed according to the methods described by Hansen et al. (1993) but with minor modifications (Watanabe et al., 2000, 2002, 2004). The BrdU-labeled cells were washed with cold phosphate-buffered saline, fixed in 70% ethanol for 60 min, pelleted and resuspended in 70% ethanol at 4 °C. Cells were then resuspended in a staining buffer (Watanabe et al., 2000, 2002) and incubated 30 min at room temperature. Cells were fractionated into six groups on the basis of cell-cycle phase, G1, S1 through S4 and G2/M, with an EPICS Elite Cell Sorter (Beckman Coulter). Equal numbers of cells (4×10^4) were collected in microfuge tubes containing lysis buffer and then incubated for 2 h at 50 °C. To provide controls for recovery of BrdU-labeled DNA, a mixture of [^{14}C] thymidine-labeled (5×10^4 dpm) Chinese hamster ovary cell (CHO) DNA and BrdU- and [^{3}H] deoxycytidine-labeled (5×10^4 dpm) CHO DNA was added to each fraction. DNA samples were purified by phenol/chloroform extraction and ethanol precipitation and dissolved in 460 μl TE containing sheared salmon testis DNA (0.5 mg/ml). The mixture was then sonicated into fragments with an average size of approximately 1 kb. These fragments were heat denatured for 3 min at 95 °C and cooled on ice. After addition of 56 μl 10× immunoprecipitation buffer (Watanabe et al., 2000, 2002) and 80 μl 25 μg/ml anti-BrdU mouse monoclonal antibody (Becton-Dickinson Immunocytometry), the samples were incubated at room temperature for 20 min with constant rotation. Antibody-bound BrdU DNA was precipitated by addition of 15 μl 2.5 mg/ml anti-mouse IgG (Sigma), and the mixture was incubated for 20 min at room temperature. After centrifugation, the pellet was washed with 1× immunoprecipitation buffer, resuspended in 200 μl digestion buffer (Watanabe et al., 2002) and incubated overnight at 37 °C. An additional 100 μl digestion buffer was added, followed by overnight incubation at 37 °C. The sample was subjected to phenol/chloroform extraction, and after addition of 20 μg yeast tRNA, DNA was precipitated with ethanol and dissolved in TE. The recovery and purity level of the BrdU-DNA was checked by monitoring the [^{3}H] and [^{14}C] counts of the CHO DNA (Tenzen et al., 1997; Watanabe et al., 2002).

2.2. PCR-based quantification of replicated DNA

Quantitative PCR was used to examine the replication timing of individual loci (Watanabe et al., 2000, 2002). Locus-specific primers were selected according to the following criterion: a single PCR product of the predicted size was amplified from THP-1 or Jurkat genomic DNA but not from CHO DNA. The primer pair for a loci was added to a single tube containing a constant amount of BrdU-labeled DNA from each cell-cycle fraction, which had been calibrated on the basis of the [^{3}H] count as described previously (Tenzen et al., 1997; Watanabe et al., 2002). In addition to a locus-specific primer, each reaction contained a constant amount of plasmid pKF3 DNA and pKF3-specific primers for assessment of PCR efficiency (Watanabe et al., 2000, 2002). The reaction buffer contained 0.5 U AmpliTaq Gold (Applied Biosystems) in 100 μl of the manufacturer's reaction buffer. Amplification conditions were one cycle of 95 °C for 9 min to activate the AmpliTaq Gold followed by 32 cycles of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min and a final cycle at 72 °C for 10 min. Gel electrophoresis and FluorImager-normalized quantification were performed as described previously (Watanabe et al., 2000, 2002, 2004). Replication timing of each locus was assigned after quantification of at least three independent PCR products from reactions in which the annealing temperature, the ratio of BrdU-labeled DNAs to pKF3 DNA and the locus-specific primer pair were altered.

2.3. Assignment of replication timing during S phase

The amount of locus-specific PCR product was normalized to that of the plasmid-specific PCR product. Replication timing was measured with a fluorescence scanning system (Fluoroimager SI, Molecular Dynamics) and assigned on the basis of six cell-cycle fractions (G1, S1–S4 and G2/M) based on the highest amount of replicated DNA after normalization to the amplified plasmid DNA. When the difference in the amount of replicated DNA between two consecutive cell-cycle fractions was less than 10%, the replication timing was defined as an intermediate period (e.g. S2.5 is between S2 and S3).

2.4. Visualisation of GC% distribution

The GC% distribution of each sequence obtained from UCSC (http://genome.ucsc.edu/) was calculated with a 500 kb window and a 50 kb step. Undetermined bases (Ns) were omitted from the GC% calculation, but the nucleotide positions were not changed. If the total of undetermined bases in a window exceeded half of the window size, the data point was omitted and the neighboring data points were connected by a line.

3. Results

3.1. Replication timing and GC content of 11q and 21q regions containing oncogenes or tumor suppressor genes in two human cell lines

Replication timing was measured as described previously (Watanabe et al., 2002, 2004, 2008). Briefly, newly replicated DNA from human cells was classified into four fractions (S1 to S4) during S phase: S1, S2, S3 and S4 correspond to very early, early, late and very late S phase, respectively. The fractions were quantified for a selected number of sequence-tagged sites (STSs) using a PCR-based method (Hansen et al., 1993) (Supplementary Fig. 1A). The timing of replication (S1 to S4) for each locus was assigned on the basis of the fraction where the largest amount of PCR product, representing newly replicated DNA, was detected. A constant amount of plasmid DNA was included in each PCR reaction to enable the band intensity in each zone to be normalized (See Materials and methods). Examples of electrophoretic patterns for the loci analyzed in this study are presented in Supplementary Fig. 1B.

In the present study, we measured replication timing of 15 known oncogenes and tumor suppressor genes on human chromosomes 11q and 21q using two human cell lines (THP-1 and Jurkat). Many of the genomic regions in which these genes located showed similar replication timing between the two cell lines, and mainly replicated intermediately between early and late S-phase in both cell lines (Fig. 1A). The replication timing of these genes and the sequences of primer sets used are shown in Supplementary Table 1. For example, the replication timing of oncogenes and tumor suppressor genes in THP-1 cells were mostly early (S2) or intermediately between early and late S phase (S2.5) (Fig. 1A). In addition, some oncogenes replicated late (S3) and very late (S3.5). In Jurkat cells, the replication timing of oncogenes and tumor suppressor genes were mostly early (S2), intermediately between early and late (S2.5), or late (S3) S-phase (Fig. 1A). Eight out of these 15 known oncogenes/tumor suppressor genes were located in GC-rich (50–55 GC%) regions, and none of the