Short Report

Identification of Human Chromosome Segments that Have High Homology with Rat Genomic DNA

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ABSTRACT

This study was conducted to determine the location of DNA segment with homology to the rat conserved genomic DNA in human chromosomes. The labeled rat genomic DNA was hybridized with normal human (male) metaphases. The study of 74 metaphases after fluorescence *in situ* hybridization showed 371 twin-spot signals on human chromosomes. Statistical analysis indicated that the specific accumulation of signals on 1q22-qter, 2p2, 3p21-p23, 4q3, 6q2, 8p12-pter, 11p12-pter, 11q12-qter, 12q2, 13p, 15p, 16q2, 21q12-qter, Yq1-qter, and Xq2 was not random. Results of stepwise multiple linear regressions indicated that number of mapped oncogenes (Beta = 1.092; t = 7.552; *P*<0.001) and density of mapped oncogenes on chromosomes (Beta = -0.832; t = -5.751; *P*<0.001) have significant effects on number of double-spots on human chromosomes. These data reflects the evolutionary conservation between rat DNA and human DNA at the above-mentioned bands. *Iran. Biomed. J. 9 (1): 37-40 2005*

Keywords: Human chromosomes, Rat DNA, High homology, FISH method

INTRODUCTION

Rat is widely used as an experimental animal in many fields of biological and medical research. Therefore, knowledge of the chromosomal location of genetic markers is highly desirable. In rat, gene-mapping studies have progressed rapidly since the early 1990s [1]. The construction of comparative maps provides an evolutionary context for interpreting mammalian genome organization during the course of evolution. Heterologous chromosome, painted with human chromosome-specific libraries, has made it possible to provide a global framework of conserved chromosomal segments among human and mammalian including cattle, goat, and horse [2-6]. As comparative mapping data of functional genes increased, it was revealed the existence of several evolutionary conserved syntenic chromosomal segments between human and rat [1]. Also, data from rat and human DNA sequencing revealed that there are many genes with high level of conservation during the course of evolution. By comparing these two sets of data it is possible to reveal the relationship between rat DNA and human chromosomes. There is no direct evidence showing where the conserved genomic DNA between rat and human located on human chromosomes. Therefore the present study was done.

MATERIALS AND METHODS

Chromosome preparation and probe. Chromosome preparation was made from a male human peripheral blood cell culture using standard cytogenetic procedure.

The probe used in this study was the Wistar rat genomic DNA. The rat genomic DNA was labeled with biotin-16-dUTP (Boehringer-Mannheim, Germany) using a nick translation kit (Boehringer-Mannheim), according to the supplier's instructions.

Fluorescence in situ hybridization (FISH). The procedure used for FISH method was previously described [7] with minor modification. In brief, chromosomal DNA was denatured by treatment in 70% formamide, 2 × SSC (1 × SSC contains; 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at 70°C for 2 min, followed by dehydration in ethanol. The slides were incubated with denatured 900 ng DNA labeled probe in a humidified box at 37°C for 18 h. The hybridization mixture was 40 µl/slide of 50% formamide, 6 × SSC, 10% dextran sulphate, and 1% BSA containing the denatured labeled DNA probe and human CotI DNA. Following hybridization, the slides were washed in 40% formamide, 6 × SSC, at 37°C for 15 min, and then washed in 2 × SSC, and 1 × SSC, respectively at room temperature for 15 min. After washing, the slides were incubated with avidin-FITC at 37°C for 60 min, amplified by incubation (37°C, 45 min) with biotinylated anti-avidin D antibodies and avidin-FITC (37°C, 30 min), and then counterstained with propidium iodide.

After FISH, metaphases with hybridization signals were photographed and the microscope stage coordinates of each metaphase were recorded. Slides were de-stained, the chromosomes were Q-banded with quinacrine and Hoechst 33258 double staining, and the same metaphases were re-photographed.

Statistical analysis. The method of Tai *et al.* [8] was used in order to evaluate the non-randomness of double-spot signal distribution on each chromosomal band(s). The relative width of each band was measured using the diagram of the International System for Chromosome Nomenclature (ISCN 1981) [9]. Multiple regression analysis and Pearson correlation coefficient analysis were used using SPSS soft ware (version 10.5). A probability of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Hybridization with the rat genomic DNA probe yielded several double-spot signals on human chromosomes. Analysis of 74 human metaphases after FISH showed 371 twin-spot signals on human chromosomes. Figure 1 shows the distribution of these signals on chromosomes. Testing the non-randomness of double-spot signal distribution at each chromosomal band is an essential step.

Statistical analysis indicated that the specific accumulation of signals on 1q22-qter, 2p2, 3p21-p23, 4q3, 6q2, 8p12-pter, 11p12-pter, 11q12-qter, 12q2, 13p, 15p, 16q2, 20q 12-qter, Yq1-qter, and Xq2 was not random.

Fig. 1. Distribution of 371 double-spot signals on human chromosomes after analysis of 74 metaphases. FISH was performed using denatured rat genomic DNA probe as described in Materials and Methods section. Each circle represents a double-spot signal.

Table 1. Analysis of number of twin-spots on chromosomes by multiple linear regressions model.

Source of variation	B	SE	Beta	t	*P* value
Constant	11.994	1.717	-	6.985	0<0.001
Number of mapped oncogenes	1.886	0.250	1.092	7.552	0<0.001
Density of oncogenes on chromosomes	- 40.846	7.103	- 0.832	- 5.751	0<0.001

F = 29.310; df = 2, 21; $P<0.001$; R square = 0.736; B, partial regression coefficient; SE, standard error; Beta, Standard coefficient.

Several reports support the view that genes are not uniformly distributed along mammalian chromosomes but are concentrated in certain regions, mainly corresponding to Giemsa pale-staining [10-12]. For example, oncogenes are scattered throughout the genome, but they tend to cluster at G-light chromosome bands [13] and most of them have a tendency to be distributed near the telomeres [14, 15]. G-negative bands contain GC-rich Alu repeats and are constitutionally more relaxed and unfolded during transcription than G-positive bands. In mitotic chromosomes, early replicating chromatin domains give rise to Giemsa light bands, whereas middle-to-late replicating domains form Giemsa dark bands and C-bands [16]. Although the relative total length of light- and dark-band is approximately equal [13], in the above-mentioned segments, total lengths of light-band increased to 59 percent of the total lengths of human chromosomes.

At least 170 oncogenes are currently listed in the human genome database. Pearson correlation coefficient analysis showed that there were significant correlations between number of double-spot signal and either chromosomal lengths (r = 0.818; n = 24; $P<0.001$) or number of mapped oncogenes on chromosomes (r =

0.566; n = 24; $P = 0.004$). Also there was a significant coefficient between number of mapped oncogenes and chromosomal lengths (r = 0.568; n = 24; $P = 0.004$). In order to rule out the effect of chromosomal length on the frequency of FISH signals on chromosomes, the stepwise multiple linear regression analysis was done using number of mapped oncogenes, density of oncogenes on chromosomes, and the lengths of chromosomes, as independent variables. The results of analysis are given in Table 1. These results indicated that a number of mapped oncogenes (Beta = 1.092; t = 7.552; $P<0.001$) and density of mapped oncogenes on chromosomes (Beta = -0.832; t = -5.751; $P<0.001$) have significant effects on number of double-spots while chromosomal length was not inter into the model. This model has determination coefficient of 0.736 (F = 29.310; df = 2, 21; $P<0.001$). Therefore, not only accumulations of double-spots on some chromosomal segments were not random, but also there are a significant correlation between number of oncogenes located on each chromosomes and number of scored twin-spot signals observed on those chromosomes.

Taken together, the present data reflect the evolutionary conservative between rat DNA and human DNA at the above-mentioned bands. It should be mentioned that it is reported the chromosome territory is evolutionary conserved during the course of higher primates evolution [17]. Although it is premature to draw a final conclusion, it is suggested that some highly conservative genes, such as oncogenes, might be located on these bands.

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