GENETICS

Stability of Minisatellite Loci in the Genome of Cultured HeLa Cells

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The stability of minisatellite loci representing DNA with the tandem organization of repeats is studied. DNA profiles of 194 HeLa clones are identical to the parent culture. They exhibit 36 bands detectable by the minisatellite probe Red4. Since the method ensures the record of changes in minisatellite loci appearing during the first two divisions of parent cell, mutations are assessed in 27,936 locus tests. Hence, the incidence of mutations in the culture is not higher than 3.5×10^{-7} per locus/per mitosis, providing that the number of generations in parental homogenous culture is taken into account.

Key Words: *HeLa; cell culture; mutations; minisatellites*

Hypervariable regions of minisatellites in genome are represented by numerous families of repeated DNA with the tandem organization of repeats. The minisatellite families differ from each other by nucleotide sequences and the length of repeat. Minisatellite loci are characterized by pronounced polymorphism as a result of differences in the number of repeats in alleles [10]. High polymorphism of minisatellite loci in a population is maintained by extremely high incidence of mutations, on the one hand, and a relative neutrality of these mutations, on the other. The frequency of mutations varies from 0.1% to several percent per gamete for different loci [3,7-9].

Recent discovery of hereditary diseases caused by triplet expansion [5], elucidation of the causes of hereditary predisposition to colon cancer associated with instability of microsatellites [6], and the relationship between the status of the minisatellite locus in the insulin gene region and the predisposition to diabetes mellitus [4] indicate functional significance of the minisatellite DNA in genome.

However, more evidence on the functional significance of minisatellites is required, which can be provided by evaluating the stability and mutability of minisatellite loci in somatic cells.

MATERIALS AND METHODS

HeLa cultures were obtained from the Laboratory of Growth Media, Institute of Carcinogenesis, Cancer Research Center, Russian Academy of Medical Sciences.

Clones were obtained by seeding the cells in Petri dishes 10 cm in diameter at a density of about 100 cells per dish in 10 ml of Eagle's medium with 20% bovine serum. Four weeks later, 194 colonies were transferred into 24-well plates (one colony per well) and cultured for 1-2 weeks, depending on the rate of clone growth. The clones were then harvested by trypsinization, seeded on 10-cm dishes, and cultured for 30 days until confluence.

For isolation of DNA from the clones and parent culture, cells were lysed in dishes with a buffer

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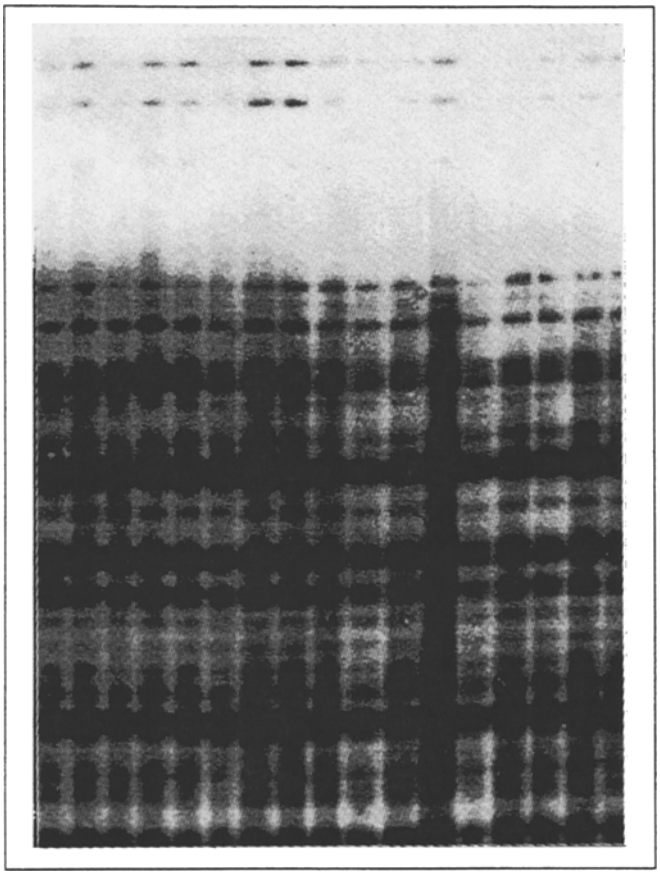

Fig. 1. Identity of DNA profiles of individual clones of cultured HeLa cells. Hybridization with the multilocus minisatellite probe Red4.

containing 1% sodium dodecyl sulfate (SDS) and 0.3 mg/ml proteinase K. The lysate was incubated overnight at 37°C, and DNA was purified by the phenol-chloroform method.

DNA was hydrolyzed with BsuR I restrictase according to the manufacturer's instructions, electrophoresed in a 0.7% agarose gel at 1 V/cm for 48 h, and transferred onto Hybond N nylon membranes (Amersham). Hybridization with the multilocus minisatellite probe Red4 [1,2] labeled with [α-^{32}P]dATP was carried out by the accidental challenge method overnight in 0.5 M phosphate buffer with 7% SDS and 1% bovine serum albumin. The filters were washed under stringent conditions (0.1% SSC, 0.1% SDS, 60°C). After washing, they were dried and exposed on an x-ray film for 1 to 7 days.

RESULTS

DNA profiles of parent culture and its 194 clones were analyzed. Each specimen contained 36 readily discernible bands available for analysis. A complete identity of DNA profiles of all clones and of the parent culture was revealed. No individual band was lost or appeared, nor did the brightness of the bands change appreciably in any clone (Fig. 1).

The sensitivity of the method is sufficient for reliable detection of 20% admixture of foreign DNA in the examined specimen. Consequently, the method detected changes in minisatellite loci (heterogeneity of the parent culture), these changes manifesting themselves during several initial divisions of the parent cell generating a clone, i.e., the clone sample (194) can be increased almost 4-fold. Therefore, the stability of minisatellite locuses was assessed in 776 cells.

Previously, we demonstrated that one band corresponded to one locus in the majority of bands in the Red4 DNA profiles [1]. The majority of Red4 minisatellite loci are heterozygous, and each locus available for radioautography analysis is generally represented only by one homologous allele due to the difference in the length of alleles [1].

Thirty-six bands (loci) were detected for each clone, hence, the total sampling of locus tests was 27,936 (776×36). Mutations were not detected. Taking the number of generations of the parent culture before cloning to be equal to 100 (which is obviously underestimated), we can claim that the frequency of mutations of Red4-minisatellite loci in HeLa cells is not higher than 3.5×10^{-7} per locus/per mitosis. This agrees with the mutation rates for the minisatellite loci in normal endothelial cells of the alimentary canal [3].

Our findings indicate a sufficiently high stability of minisatellite loci in cultured HeLa cells, which reflects the significance of these loci for normal functioning of genome and is provided by the mechanisms maintaining their stability. This agrees with the inclination of these cells to accumulate chromosomal rearrangements, because the stability of genome at different structural levels is likely to be maintained by different mechanisms. The frequency of mutations in germ cells for the Red4 loci is 8.1×10^{-3} per band/per infant [1]. The frequency of mutations in the minisatellite loci in male and female germ cells is virtually the same, irrespective of different number of divisions before maturation of oocyte spermatozoid. The fact that the frequency of mutations in germ cells is much higher than in somatic cells requires further analysis. High rate of minisatellite mutations in germ cells and the stability of minisatellites in somatic cells apparently indicate that the majority of inherited mutations *de novo* occur in meiosis during crossing-over. However, other studies demonstrate that unequal crossing-over is hardly responsible for inherited *de novo* mutations in minisatellite loci [11].

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Specific Features of Gene Expression in Human Myoblasts. Analysis of Cells from Primary and Cloned Cultures

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Primary cultures of postnatal human myoblasts are obtained. Their purity is assessed by cytochemical determination of alkaline phosphatase activity and electrophoretic analysis of the expression of muscle proteins in comparison with postnatal human fibroblasts.

Key Words: *human myoblasts; alkaline phosphatase; tissue-specific muscle proteins*

Cultured mononuclear muscle cells (myoblasts, MB) obtained from various organisms are a convenient model for the investigation of cell differentiation and other biological processes [8,12,13]. So far, cultures initiated from rat or mouse muscles provided essentially stable lineages [11,12]. Meanwhile, human MB are of specific interest. In addition, considerable attention has been focused on these cells after successful cellular or gene therapy of Duchenne's muscular dystrophy by grafting donor MB in patient's muscles [8,9]. Donor's cells fused with patient's multinuclear myofibrils, which triggered the synthesis of dystrophin in patient's myofibrils.

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The presence of fibroblasts (FB) whose content varies in a wide range hampers the use of primary MB cultures as a model system in experimental studies and as a graft in cellular therapy.

We studied some specific features of gene expression in human MB and evaluated some protein markers for the determination of the MB/FB ratio in primary cultures.

MATERIALS AND METHODS

Primary MB cultures were initiated from human muscle specimens (1 g) obtained after treatment of the operative field. The tissue was washed with normal saline containing 1000 U/ml ampicillin and 200 U/ml streptomycin and treated with 0.1% collagenase (Sigma) and 0.1% trypsin for 1 h at 37°C. Cells

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