THE NATURAL MUTATION PROCESS IN HUMAN DIPLOID STRAINS

N. V. Chervonskaya and R. I. Rapoport*

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The level of natural mutation in cells of human diploid strains varies with the duration of their cultivation. In the first and second phases of growth it is low and indistinguishable from the level of spontaneous mutagenesis in cells of a primary culture of human embryonic tissue and a culture of human circulating blood leukocytes. Mutation is appreciably intensified in the third, degenerative phase of growth of the culture. Spontaneous chromosomal aberrations arise at all stages of the mitotic cycle, but at different frequencies. They are formed in the largest number in the postsynthetic stage, with the resulting predominance of aberrations of chromatid type.

The use of cells of human diploid strains to prepare vaccines against viruses requires constant karyologic control over the strains. For this purpose it is necessary to know the level of inherited mutation of these cells under normal conditions throughout the period of cultivation. In addition, the study of natural mutagenesis in diploid cells is interesting as an aid to the understanding of aging in the organism, for the properties of these cells and of human cells in vivo are quite close.

The level of natural mutation in cells of human diploid strains increases in the third phase of their growth [13, 17]. However, information regarding natural mutation in the cells in the first and second phases of growth is inadequate and contradictory. No information is available for the dynamics of genome mutations. The existing data for chromosomal aberrations in these cells is of a general nature, and the aberrations discovered have not been classified into types.

Accordingly the present investigation was carried out to study spontaneous structural changes in the chromosomes and genome mutations in cells of human diploid strains in the three phases of their growth.

EXPERIMENTAL METHOD

Experiments were carried out on cells of several diploid strains: strain WI-38 (obtained from Dr. L. Hayflick at the Philadelphia Institute of Anatomy and Biology), and strains L-51, L-52, and L-53 (isolated in the writers' laboratory). All strains were isolated from the lung tissue of 3-4-month human embryos. The methods of cultivating the cells, of making the specimens of cytogenetic investigation, and of statistical analysis of the results have been described previously [11]. Chromosomal aberrations were studied in the metaphase of mitosis. For the analysis of the commonest terminal chromatid ruptures, the schemes of Rivell as modified by Demin et al. [3] were used. In the analysis of genome mutation, hypoploid sets with a chromosome number less than 45 were regarded as technical losses and were disregarded [1, 9]. In each experiment 100-150 metaphases were analyzed. The number of polyploid cells was determined by counting 250 metaphases irrespective of the degree of divergence of the chromosomes with an accuracy of ±10 chromosomes.

*Deceased.

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TABLE 1. Number and Types of Spontaneous Mutations of Chromosomal Structure in Cells of Human Diploid Strains in Different Phases of Growth

Phase of growth	Number of cells studied	No. of cells with chromosomal aberrations		Type of chromosomal aberrations				No. of cells with achromatic deficiencies	
		abs.	%	chromatid ruptures	chromatid translocations	isochromatid and chromatid ruptures	asymmetrical chromosomal translocations	abs.	%
First	411	5	1.2±0.5	5	—	—	—	8	1.9±0.7
Second	749	17	2.3±0.5	15	—	2	—	17	2.3±0.5
Third	335	16	4.7±1.1	9	1	4	3	7	2.1±0.8

TABLE 2. Number of Genome Mutations in Cells of Human Diploid Strains in Different Phases of Growth

Phase of growth	No. of cells studied	Percentage of cells with number of chromosomes given below			Percentage of tetraploid cells
		45	46	47	
First	315	16.5±2.1	82.2±2.1	1.3±0.6	1.2±0.5
Second	617	17.6±1.5	79.7±1.6	2.7±0.6	0.9±0.2
Third	262	23.3±2.6	69.2±2.8	7.5±1.6	4.7±0.8

EXPERIMENTAL RESULTS

In the investigation of the cells of different strains and different substrains of strain WI-38, propagated after storage in liquid nitrogen and cultivated at different times, no statistically significant difference was found in the number of chromosomal aberrations ($P < 0.95$), so that pooled data are given in the tables. Altogether 1495 metaphases were studied: 411 in the first phase, 749 in the second, and 335 in the third phase of growth (Table 1). According to these results the level of natural mutation in cells of human diploid strains increases with an increase in the duration of their cultivation. Whereas the number of cells with chromosomal aberrations in the first phase of growth was 1.2%, and in the second 2.3% (difference not statistically significant, $P < 0.95$), in the third phase it reached 4.7% at the level of the passages studied (up to the 35th passage; difference statistically significant, $0.95 < P < 0.99$). In cells in the first phase of growth, all the changes found in the chromosomes were aberrations of chromatid type and consisted of terminal chromatid ruptures. In the cells of the second phase of growth, there were about 90% of terminal chromatid ruptures, and two aberrations belonged to the group of undifferentiated changes (isochromatid and chromosomal ruptures). In the cells in the third phase of growth, with an increase in the total number of aberrations, aberrations of both chromatid and chromosomal types were found, although the former were predominant (by 2.5 times). It was accordingly concluded that structural mutations in chromosomes in cells of human diploid strains arise at different periods of the mitotic cycle, and not only in the postsynthetic period, as has hitherto been considered [5, 18]. However, the largest number of chromosomal mutations arises in the postsynthetic period of the mitotic cycle, and this results in predominance of aberrations of the chromatid type.

The number of cells with achromatic deficiencies did not change with the age of the strains (Table 1), and no rule governing their appearance could be detected.

With an increase in the period of cultivation, an increase in the number of genome mutations was found in the cells of the human diploid strains (Table 2). Whereas the numbers of hypoploid and hyperploid cells (aneuploid genome mutations) were 16.5 and 1.3%, respectively, in the first phase, and 17.6 and 2.7% in the second phase, in the third phase they showed an increase to 23.3 and 7.5%. The difference in the number of these cells in the second and third phases of growth is statistically significant. The level of significance for the hypoploid cells $t_{diff} = 2.7$, and for the hyperploid cells $0.999 > P > 0.99$.

The number of polyploid cells (euploid genome mutations) in cultures in the third phase of growth was increased to 4.7%, whereas in the first and second phases it was 1.2 and 0.9%, respectively. The difference is statistically significant (> 0.999).

Evidence for an increase in the level of natural mutation with an increase in the period of cultivation of cells of human diploid strains is thus given by an increase in the number of chromosomal and genome mutations in the cells in the third phase of growth. These changes can be compared with those taking place in human circulating blood cells during aging [6, 12, 14-16], although the level of natural mutation in the cells of human diploid cells in the third phase of growth rises more rapidly than in the blood cells of old people. On the other hand, the number of chromosomal mutations in cells of human diploid strains in the first and second phases of growth is small (1.2-2.3%) and does not exceed their number in the cells of a primary culture of human embryonic tissues or a culture of human circulating blood which, according to some authorities, lies between 1.2 and 2.5% [2, 4, 7, 8, 10].

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