GENETICS

Cytogenetic Characteristics of T-Lymphoid Cell Strains of *Papio Hamadryas*

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A detailed cytogenetic study of three T-lymphoid strains of *Papio hamadryas* cells was carried out. Two strains, LNPH-5(T) and SPH-7(T), had normal karyotype 42,XX at the 43rd and 56th passages. After long culturing the initial SPH-7(T) cells were completely replaced by the 87th passage by cells with a pseudodiploid karyotype 42,XX,der(1), der(2),der(13),der(14),der(X). SPH-8(T) strain (36th passage) contained two cell clones: with predominant normal (42,XY) and pseudodiploid 42,XY,der(7)t(7;X)(qter;pter), del(13)(q21),del(14)(q16) karyotype. The patterns of karyotypic variability of simian and human T-lymphoid cell strains are similar.

Key Words: *T-lymphoid cell strains; Papio hamadryas; karyotype; chromosome variability*

Human and animal T-cell strains are isolated much more rarely than B-lymphoid strains [2,10]. All human T-lymphoid cell strains contain HTLC virus and the majority are nontumorous, despite their source (tumorous or normal hemopoietic tissues) [9]. Long culturing of such cells *in vitro* may lead to evolution of cell karyotype [9,10], and the developing secondary structural changes in chromosomes are similar to specific chromosome rearrangements in human T-cell lymphomas and leukemias [12].

T-Lymphoid cell culture was derived from *Papio hamadryas* with T-cell lymphomas (Sukhumi population), and a unique collection of continuous suspension T-lymphoid simian cell strains was created [1,2]. Comparison of simian and human B-lymphoid cell cultures showed similar changes in these strains' karyotypes during long culturing *in vitro* [3]. There are no published karyotypical analyses of simian T-cell cultures. We studied cytogenetic characteristics of T-lymphoid cells of *Papio hamadryas* and chromosomal changes in these cells cultured *in vitro*.

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MATERIALS AND METHODS

The following suspensions T-lymphoid cell strains derived from *Papio hamadryas* were studied: LNPH-5(T), SPH-7(T), and SPH-8(T) containing simian T-lymphotropic virus type I (STLV-1). All the strains were from the Sukhumi Institute of Experimental Disease and Therapy and were initiated from animal hemopoietic tissues with T-cell lymphomas: LNPH-5(T) from the lymph node cells and SPH-7(T) and SPH-8(T) from splenic cells [1,2]. Cells were cultured in RPMI-1640 with 15% fetal calf serum (Flow), L-glutamine (300 mg/ml), human recombinant interleukin-2 (100 U/ml), penicillin (100 mg/ml), and streptomycin (50 mg/ml).

The metaphase plate slides were routinely prepared from human cell cultures 48 h after reino-

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culation. The cells were treated by a hypotonic solution of 0.56% KCl and 1% sodium citrate (1:1) for 10-20 min at 37°C and fixed in a methanol-glacial acetic acid mixture (3:1). Cell suspension was layered onto cooled slides and dried in the air or by burning the fixator. Cytogenetic analysis was carried out as recommended previously [5]. Chromosomes were counted in preparations stained according to Giemza and air-dried. The variability of the chromosome number, modal number of chromosomes, and percentage of cells with modal chromosome number were estimated on 100 metaphase plates with intact cytoplasm. The percentage of polyploid cells was determined in a sampling of 1000 metaphase plates. For detailed karyotypical analysis, the chromosome preparations were dried by burning the fixator and stained for G disks by a previously described method [4]. Normal chromosomes, the origin of structurally altered (marker) chromosomes, and typical karyotypes of strains were identified by microphotographs of 15 metaphase plates. Simian chromosomes were identified in accordance with the nomenclature [6].

RESULTS

The modal chromosome number in the diploid chromosome set 2n=42 was retained in all strains (Table 1). The variability of the chromosome number was no more than 2-7 chromosomes. The highest percentage of cells with modal chromosome number (95%) was observed in LNPH-5(T)strain (56th passage). In SPH-8(T) (36th passage) and SPH-7(T) strain (43rd passage), the counts of cells with the modal chromosome number were similar (60 and 70%, respectively). Further culturing of SPH-7(T) led to a decrease in the percentage of cells with the modal chromosome number to 52% by the 87th passage. Studies of human lymphoid strains showed that high stability and intact chromosome disomy during the first year of culturing are typical of the majority of strains [5,10]. Our results confirm that this feature is also characteristic of simian lymphoid cells. The content of polyploid cells (as a rule, tetraploid) was sufficiently high in all cell strains and approximately the same (11.8-17%). High ploidy of cells in cell cultures is believed to indicate that their *in vitro* development is in progress and may continue to 100-150 passages [5]. Other scientists think that polyploid development of cells is a method by which cells compensate for the genetic defects and adapt to culturing [5].

Cytogenetic analysis of T-lymphoid cells of *Papio hamadryas* showed that at early passages, all cells in SPH-7(T) and LNPH-5(T) strains have normal karyotype, cells with normal karyotype predominating in SPH-8(T) (60%). These data indicate that all studied cell strains are lymphoblastoid (nontumorous), because lymphoid cells derived from nontumorous cells of tumor preparations possess a stable normal karyotype at early stages of culturing, and cell heterogeneity is acquired in the course of culturing [10]. Cells with two karyotypes, normal and pseudodiploid, which contained an extracopied marker chromosome t(7;X)(qter;pter) and deletions of chromosomes 13 and 14, were detected in SPH-8(T) strain as early as during the 36th passage. In SPH-7(T), initial cells with normal karyotype were completely replaced by cells with polyploid karyotype with chromosomes 1, 2, 13, 14, and X structurally altered by the 87th passage (Fig. 2).

The identity of the patterns of differentiated staining of chromosomes belonging to *Papio* and *Macaca* has been proven [13]. The genetic material in human and *Macaca* chromosomes is completely homologous [14]. This prompted the comparison of chromosomal rearrangements in T-lymphoid cells of *Papio hamadryas* and man; we failed to find reports about karyotypical analysis of simian T-cell cultures. Thus, *Papio hamadryas* chromosomes, which are involved in chromosome transformations during culturing of T-lymphoid cells, are homologous to

TABLE 1. Karyotypes of *Papio Hamadryas* T-Lymphoid Cell Strains

Strain	Passage	Modal chromosome number	Percentage of cells with modal chromosome number	Karyotype	Polyploidy, %
SPH-7(T)	43	42	72	42,XX	13.0
	87		52	42,XX,der(1),der(2),der(13),der(14),der(X)	—
SPH-8(T)	36	42	60	42,XY/42,XY,der(7)t(7;X)(qter;pter),del(13)(q21),del(14)(q16)	11.8
LNPH-5(T)	56	42	95	42,XX	17.0

Note. Dash: not studied.

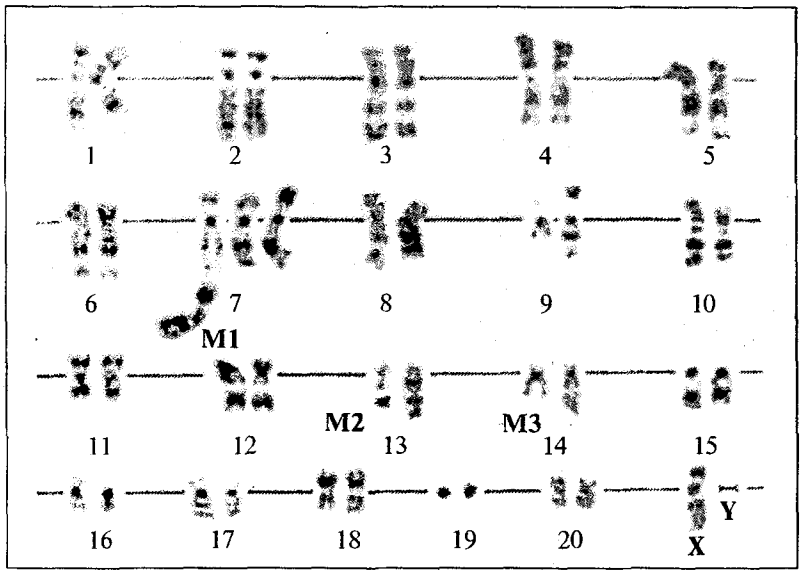

Fig. 1. Karyotype of SPH-8(T), 42,XY; 3 marker chromosomes. Here and on Fig. 2: G-staining, ×700.

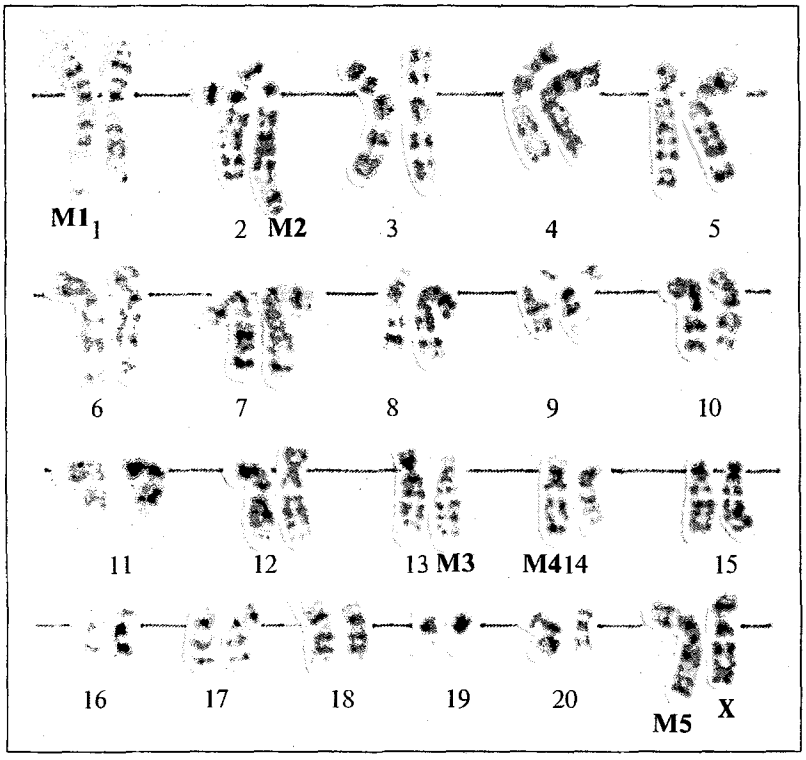

Fig. 2. Karyotype of SPH-7(T), 42,XX; 5 marker chromosomes.

the following human chromosomes: *Papio* chromosome 1 to human chromosome 1, chromosome 2 to human chromosomes 7 and 12, chromosome 7 to human chromosomes 14 and 15, chromosomes 13 and 14 to long and shorts arms of human chromosome 2, and X-chromosome to human X-chromosome.

Extracopying of *Papio* chromosomes 7 and X, which are involved in the formation of marker chromosome in SPH-8(T)strain is not accidental, like a frequent increase in the number of copies of chromosomes homologous to these *Papio* chromosomes in human lymphoid tissues [10]. Little is known about the localization of unique genes, particularly of oncogenes and other growth-associated genes, in chromosomes of the lower monkey species. Specific rearrangements involving chromosome 14 are typical of many types of malignant T-cellular lymphomas in man [7,8]. Human chromosome 14 contains the genes of T-cell receptor α- and δ-chains. Chromosome 15 is involved in structural rearrangements in human leukemia [7] and bears and c-fes oncogene. Presumably, the increased number of copies of chromosomes 7 and X in the *Papio* lymphoid strain

and, hence, the doses of structural genes and gene products, impair the regulatory gene mechanisms, thus increasing the proliferative potential of cells with trisomies and ensures selective advantages for them in a culture.

The involvement of specific chromosome sites in structural rearrangements in human hemoblastosis is no longer doubted [7,8]. On the other hand, we know that long culturing of lymphoid strains is associated with the development of secondary chromosome alterations in these cells [10,11]. Besides extracopying individual chromosomes, a characteristic feature of lymphoid cell strains (and all continuous cell strains) is that structural rearrangements of chromosomes developing during their *in vitro* culturing are not accidental [5,10]. The involvement of the same chromosomes 13 and 14 in secondary structural changes in strains SPH-7(T) and SPH-8(T) in our study is of special interest. These *Papio* chromosomes together are homologous to human chromosome 2, whose structural rearrangements are specific for human T-cell tumors [7,12]. In SPH-7(T) strain, *Papio* chromosomes 1 and 2 changed by the 87th passage. Various sites of human chromosomes, homologous to these *Papio* chromosomes, are often involved in secondary chromosome restructuring in continuous human lymphoid cell strains [10-12].

Therefore, our results indicate that the evolution of karyotypes of *Papio* T-lymphoid cells of non-tumor origin in the course of their development and long culturing *in vitro* is similar to that of human lymphoid cells. The similarity consists in the involvement of chromosomes with homologous genetic material in quantitative and structural rearrangement of chromosomes.

REFERENCES

1. V. Z. Agrba, V. V. Timanovskaya, V. V. Kakubava, *et al., Eks. Onkol.*, **15**, No. 4, 38-40 (1993).
2. V. Z. Agrba, V. V. Timanovskaya, M. G. Chikobava, and L. V. Indzhiya, *Tsitologiya*, **34**, No. 9, 44 (1992).
3. D. E. Araviashvili, V. Z. Agrba, V. V. Timanovskaya, *et al., Ibid.*, **36**, No. 7, 701-707 (1994).
4. S. Yu. Demin and S. E. Mamaeva, *Ibid.*, **35**, No. 9, 18-23 (1993).
5. S. E. Mamaeva, *Ibid.*, **38**, No. 8, 787-814 (1996).
6. B. Dutrillaux, M. Muleris, and M. Paravatou-Petsota, *Mutat. Res.*, **126**, 93-103 (1984).
7. M. M. Le Beau, *Semin. Oncol.*, **17**, 20-29 (1990).
8. F. Mitelman, *Catalog of Chromosome Aberrations in Cancer*, New York (1994).
9. T. Naoe, Y. Akao, K. Yamada, *et al., Cancer Genet. Cytogenet.*, **34**, 77-88 (1988).
10. K. Nilsson, in: *The Epstein-Barr Virus*, eds. M. A. Epstein *et al.*, Berlin-Heidelberg-New York (1979), pp. 225-281.
11. M. Shade, M. A. Woodward, and C. M. Steel, *J. Natl. Cancer Inst.*, **65**, 101-109 (1980).
12. S. D. Smith, R. Morgan, M. P. Link, *et al., Blood*, **67**, 650-656 (1986).
13. R. Stanyon, C. Fantini, A. Camperio-Ciani, *et al., Am. J. Primatol.*, **16**, 3-17 (1988).
14. J. Wienberg, R. Stanyon, A. Jauch, and T. Cremer, *Chromosoma*, **101**, 265-270 (1992).