CYTOGENETIC ANALYSIS OF THE SPONTANEOUSLY IMMORTALIZED MOUSE CELL LINE G1

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Cytogenetic analysis of the spontaneously immortalized mouse cell line G1 has been carried out on passages 15, 24, 53, 68, 104, and 140 of in vitro cultivation. G1 cells were revealed to have quantitative chromosome changes, different chromosomal aberrations, and a particularly high percentage of Robertsonian translocations. The heterogeneity of cell population was observed to diminish during long-term cell cultivation. The karyotypic evolution of cells lasted not less than 140 passages on the stage of establishment.

Keywords: *karyotypic evolution; chromosome aberration; Robertsonian translocation.*

Rapid development of modern novel biotechnologies involving mammalian culture cells (including different kinds of stem cells) and their practical application in cell therapy are the essential factors of intensive cytogenetic investigations of cells in culture.

The knowledge and understanding of general cell karyotypic variability principles allows forecasting the behaviour of any particular cell line even before *in vitro* cultivation, *i.e.* on the stage of establishment. This forecasting becomes significant using permanent cell lines in both various molecular and genetic experiments and in biotechnological researches. The process of karyotypic evolution of cell line is known to consist of two qualitatively different stages, namely, the stage of establishment and the stage of stabilization [1, 2]. The first stage is known to be the least studied one as at this stage the adaptative changes of chromosomal apparatus of cell line take place, *i.e.* quantitative and structural rearrangement of chromosomes and cell population is karyotypically heterogenous. The characteristic feature of the stage of stabilization is insignificant variability of the number of chromosomes, expressed by modal class of the number of chromosomes and the karyotype balance.

The study of karyotypic variability principles, common for both *in vivo* and *in vitro* cells, is of great importance nowadays. The "hotspots"-spreading pattern on the chromosomes and change in number of some chro-

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mosomes are the morphological demonstrations of molecular and biochemical processes, connected to changes in quantity and structure of specific genes, the oncogenes in particular. This circumstance conditions the perspective for the application of cell lines in order to study the role of specific chromosome changes in malignant transformation of cells [1, 2]. The study on chromosomal instability during establishment of cell lines *in vitro* may serve as a model of spontaneous transformation of cells *in vivo*.

The purpose of current work was to investigate the cytogenetic changes, which occur in mouse stem cells of embryonic origin, in the course of long-term monolayer cell cultivation *in vitro*.

Materials and Methods. The work was performed on mouse cell line G1, obtained from a BALB/c mouse embryo material line [3, 4]. The cells were cultivated in DMEM medium (Sigma, USA), adding 100IU/ml of penicillin, 100?g/ml of streptomycin, and 5% of fetal bovine serum (Sangva, Ukraine).

*Chromosomal Analysi*s

Chromosome preparations were made in accordance to commonly accepted method [5], modified in our lab, without colchicine or any other mitotic poisons, and cells were hypotonized for 30-40 min in the deionized water, 37°C [3, 4]. The preparations were stained for 10-20 min with 5% solution of Giemsa's stain, prepared on phosphate buffer (pH 6.8). The metaphase spreads analysis was performed using Jenaval microscope (Carl Zeiss, Austria) (lenses ×40 and ×100).

Karyotypic analysis

Karyotypic analysis was performed on passages 15, 24, 53, 68, 104, and 140 of cells cultivation *in vitro.* Qualitative and quantitative chromosome changes were analyzed, *i.e.* two types of aneuploidy (hypodiploidy, chromosome number less than 40, and hyperploidy, chromosome number more than 40), euploidy, chromosomal aberrations (chromatid and chromosome breaks, fragments, ring chromosome, interchanges and translocations), Robertsonian translocations. In order to determine the modal class and variability of cells by the number of chromosomes, 100 to 1000 of metaphase spreads were analyzed in each variant.

Micropicturing

Microobjects were pictured using photo cameras *Kyiv* and *Zenit*, colour films *Kodak*-100 and *Kodak*-200, black-and-white films *Svema*-64 and *Svema*-100.

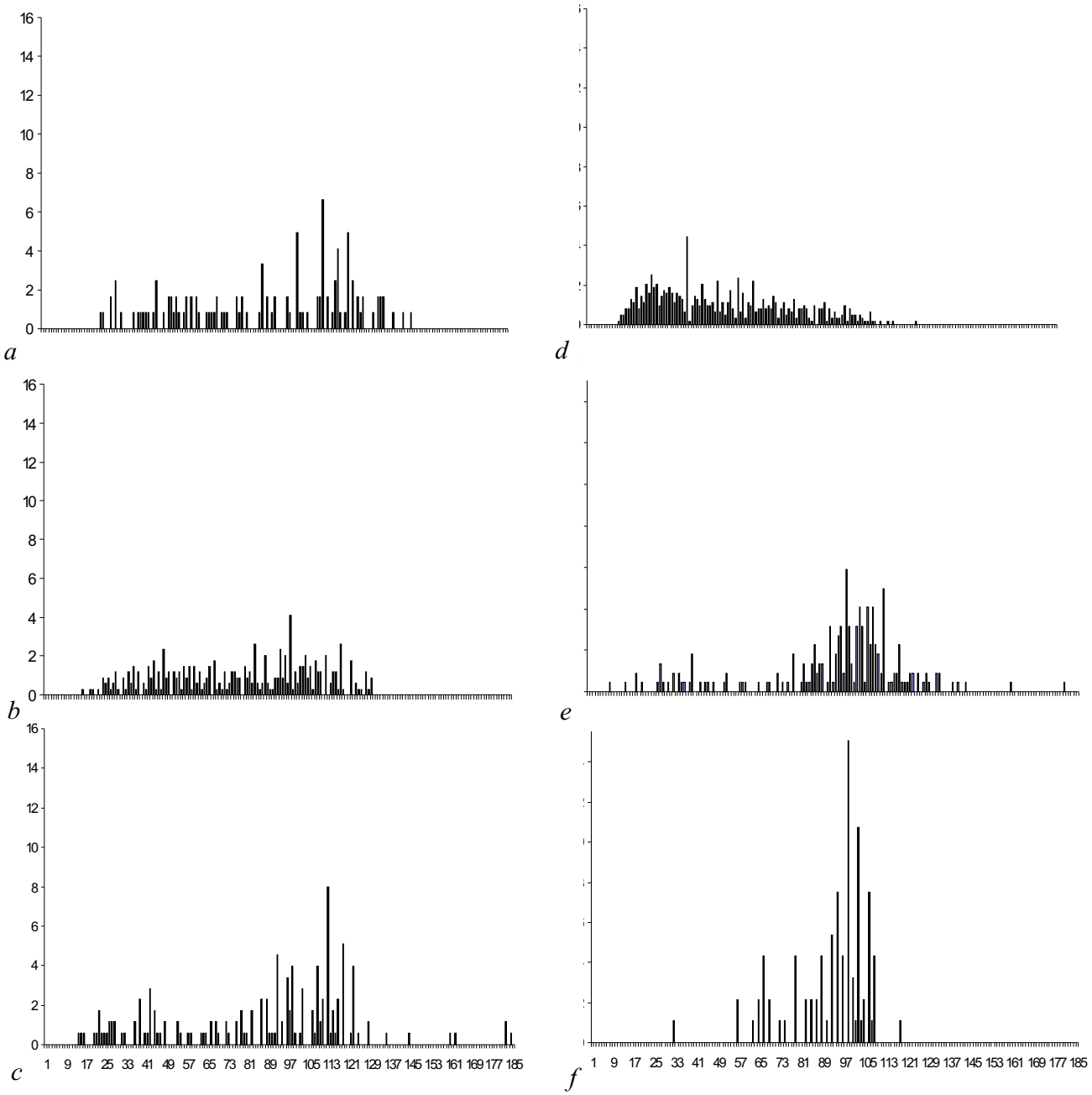
Statistical analysis

The statistic results were worked out according to Plokhinsky's method [6].

Results and Discussion. The morphological and growth characteristics of new mouse cell line G1 were studied previously by us [3, 4]. The morphological heterogeneity of the mentioned line with the dominant cell type – fibroblast-like cells – and the features of neoplastic cell transformation were revealed. The significant percentage of multinucleate cells and the cells with micronuclei, as well as different forms of aberrant mitoses, were detected in cell populations of the mentioned line, which testifies to the increased chromosomal instability of G1 cell line and cell division deregulation [4].

G1 cell line rearrangement analysis by the number of chromosomes at different stages of *in vitro* cultivation revealed significant heterogeneity of cell population and its gradual decrease in the course of long-term cultivation. In particular, the cell population of the 15th passage of cultivation was highly heterogeneous by the quantitative composition of chromosomes, which was revealed by the presence of about 15 classes of cells by a chromosome number (Fig.1, *a*). Such high karyotypic heterogeneity of cells is a specific feature of cell cultures at the earliest stages of cultivation; however, further cultivation usually results in its diminishing and isolation of modal class of chromosomes [7].

The number of chromosomes on the 15th passage varied from hypodiploids to hyperploids (containing over 100 chromosomes). The dominant clones of cells with hypodiploid (28-30), hyperdiploid (46), hypertetraploid (88), near-pentaploid (102) chromosomes, and the groups of near-hexahyploid line (112-124) can be distinguished. These six groups of dominant cells are most likely to have a tendency to form a modal class at establishment of mouse cell line G1 *in vitro*. The absence of the distinguished modal class and the modal number of chromosomes shows that the cells of the 15th passage of cultivation are still at the stage of cell line establishment.



On the 24th passage of *in vitro* cultivation population of mouse cell line G1 was revealed to be highly heterogeneous in a chromosome number, up to 20 modal classes (Fig.1, *b*). However, the number of groups, possessing the tendency to form the modal class was diminished down to 4, namely, near-diploid (42-48), near-tetraploid (80-88), near-pentaploid (94-98), and near-hexaploid (118-122) (Fig.1, *b*). It is noteworthy that on the 24th passage, as well as on the 15th passage, significant variability in the number of chromosomes (from near-haploids to hyperhexaploids with significant number of cells of near-pentaploid

Table 1.
The distribution of mouse G1 cells by the chromosome number on different passages

Passage	The number of analyzed metaphases	Number of cells, %				
		Diploid set of chromosomes (2n=40)	Euploids	Aneuploids		High diploids (2n > 100)
				Hypodiploids (2n < 40)	Hyperploids (2n > 40)	
15	121	0.83±0.83	4.13±1.82	8.26±2.51 95.04±1.98	86.78±3.09	47.93±4.56
24	345	0.58±0.41	4.06±1.06	11.59±1.73 95.36±1.13	83.77±1.99	30.14±2.47
53	176	0.57±0.57	1.14±0.80	14.20±2.64 98.30±0.98	84.09±2.76	41.48±3.72
68	635	4.41±0.82	3.31±0.71	35.43±1.90 92.28±1.06	56.85±1.97	6.93±1.01
104	221	1.81±0.90	4.07±1.33	8.14±1.84 94.12±1.59	85.97±2.34	52.04±3.37
140	93	–	3.23±1.84	1.08±1.08 96.77±1.84	95.70±2.11	33.33±4.91

Note: "–" – were not observed

group) and the absence of distinguished modal chromosome number and modal class were detected.

Mouse G1 cell population of the 53rd passage of *in vitro* cultivation had about 13 cell modal classes by the number of chromosomes in them (Fig.1, *c*). Karyotypic heterogeneity remained, however, the decrease in the number of cell groups of possessing the tendency to form modal class was observed – the dominance of cells of near-pentaploid group, among which there were 2 near-pentaploid groups (92-98 and 108-112) and one near-hexaploid group (118-122), was observed. The group of near-diplod cells (38-44) was also distinguished.

On the 68th passage of *in vitro* cultivation the change of dominant populations of near-penta- and near-hexaploid cells took place. This phenomenon is not rare during long-term cultivation [7]. The number of modal classes decreased to nine and the dominant populations were of near-haploid (18-28), hypodiploid (30-38), hyperdiploid (46-57), and near-triploid (60-66) groups having the significant advantage of cells with diploid number of chromosomes (40) (Fig.1, *d*). However, in this case the cells of greater ploidy, namely, near-tetraploid, near-pentaploid, and some of near-hexaploid groups, were also present in small amounts.

Investigating next passages, the restoration of near-penta- and near-hexaploid cell populations dominance and the decrease of heterogeneity of the cells by the number of chromosomes was observed. The number of cells with less than 80 chromosomes decreased and the rapid increase of near-pentaploid part was revealed (Fig.1, *e, f*). In particular, the 104th passage showed that near-pentaploid cells dominated in G1 cell line population and five modal classes could be distinguished (Fig.1, *e*); on the 140th passage two modal classes (number of chromosomes 94-98 and 102-108 (Fig.1, *f*)) as well as two modal numbers of chromosomes (98 and 102) were distinguished. It may be considered as the initial stabilization stage of G1 cell line.

The investigated cell line, regardless of its clonal origin, showed the karyotypic heterogeneity in the wide

Table 2.
The distribution of mouse G1 cells by the number of Robertsonian translocations (RT).

Passage	The number of analyzed metaphases	Number of cells with RT, %					
		Total	Number of RT per one metaphase spread				
			1	2	3	4	5
15	96	64.58±4.91	55.21±5.10	8.33±2.84	1.04±1.04	-	-
24	222	36.47±3.24	31.53±3.13	4.05±1.33	0.45±0.45	-	0.45±0.45
53	176	23.30±3.20	21.02±3.08	1.70±0.98	0.57±0.57	-	-
68	635	18.11±1.53	15.43±1.43	1.42±0.43	0.94±0.38	0.31±0.22	-
104	213	53.99±3.42	37.56±3.33	15.02±2.45	0.94±0.66	0.47±0.47	-
140	93	74.19±4.56	38.71±5.08	24.73±4.50	8.60±2.92	1.08±1.08	1.08±1.08

Note: "–" – were not observed

ranges and was characterized by frequent change of dominant clones. Therefore, the process of its establishment lasted long and the stabilization began at the 140th passage, comparing to immortalization of cell lines, obtained from 14 days p.c. embryos of BALB/c mouse strain, which occurred till the 20th passage, as well as with the beginning of cell stabilization, obtained during long-term *in vitro* cultivation of mouse rhabdomyosarcoma cells which occurred on passages 30-50 [7, 8].

The subdivision of cells into big groups according to ploidy revealed a significant percentage of hyperploid cells (2n > 40) on all the stages of establishment of mouse G1 cell line (Table 1). However, the fluctuations of high-ploid cells (2n > 100) with very rapid decrease of their number on the 68th passage were observed (Table 1). The dominance of near-diploid cell line and the highest number of cells having diploid number of chromosomes (app. 4% in comparison to 1% at earlier stages and their absence at the beginning of stabilization of cell line) were observed on this passage. The number of euploid cells (?20) during cultivation did not fluctuate much – some insignificant changes in their number were observed on the 53rd passage of *in vitro* cultivation, app. 1% in comparison to 3-4% on other passages (Table 1).

The significant percentage of cells with Robertsonian translocations of chromosomes was revealed in the process of *in vitro* cultivation of mouse G1 cell line population (Table 2). From one to five Robertsonian translocations (RT) per metaphase were observed (Fig.2). Thus, on the 15th passage more than 60% of cells with RT were found. Farther cultivation resulted in decrease amount of cells with RT to app. 18% on the 68th passage. However, their number increased rapidly to over 70% on the 140th passage. Therefore, high frequency of cells with RT is a specific feature of G1 cell line.

Cytogenetic anomalies due to innerchromosomal damages are known to be typical for BALB/c mouse strain, *e.g.* chromosomal aberrations and RT. Therefore, in accordance to Vagina *et al.* in bone marrow of BALB/c mouse strain, the cell line understudy was obtained from, the RT frequency amounted to app.7% [9]. Interchromosomal associations of homologous and non-homologous chromosomes according to RT are also known to be determined by the adaptation of cells to cultivation and are very convenient chromosomal markers of corresponding cell lines [10]. It is possible to suppose that the embryonic cells obtained from BALB/c mouse strain were initially biased to RT formation.

Having compared the results of chromosomal analysis of cell subdivision by the chromosome number, ploidy, and amount of RT, the conclusion can be made that high ploid G1 cells, near-pentaploid and near-hexaploid ones, in particular, RT percentage increases, *i.e.* the process of polyploidisation of mouse genome is accompanied by numerous associations of acrocentric chromosomes into metacentric ones. The

Ris2.

Ris3.

genome of the cell is trying to decrease the number of chromosomes without the loss in genetic material. At cell ploidy increase the number of various genes increases correspondingly, *i.e.* the percentage of genes,

oncogenes, in particular, increases. Due to this fact, the cells of higher ploidy are characterized by increased adaptive potential and selective advantages at *in vitro* conditions in comparison to the cells of normal karyotype.

Having investigated HyperCLDB (Cell Line Data Base hypertext; http://www.biotech.ist.unigue.it) we found out that 582 of cell lines were obtained in total, among which 111 were out of the BALB/c mouse strain (http://www.biotech.ist.unigue.it/cldb/spestr.html). Moreover, the cell lines obtained from these mice are myelogenous predominantly [11] and only an insignificant amount is fibroblast-like cultures with monolayer growth.

Among the lines, presented in the catalogue of Russian collection of cell cultures [12], according to karyotypic characteristics, their variability by the chromosome number is in the borders of 50-80 chromosomes and rarely around 60-100, some of them have got minichromosomes, chromosomal markers, *i.e.* metacentrics or submetacentrics. Structural rearrangements of chromosome 15 are marked in mouse cell lines the most frequently, and rather rare they are marked on chromosomes 1, 6, and 12 [1]. However, the most active part in RT in bone marrow cells of BALB/c mouse strain is shown for chromosomes 1, 12, and 5 [13]. Previous results of detailed karyotypic analysis of G1 cell line indicate that the tendency to dominant participation of the biggest chromosomes of mouse genome, *i.e.* 1, 2, and 3, in RT formation is observed.

Other chromosomes aberrations were revealed at the analysis of metaphase chromosome preparations of mouse G1 cells [3, 4, 14], chromatid and chromosomal breaks with formation of acentric, symmetric and asymmetric exchanges and translocations (Fig.3), in particular, as well as the rings, minichromosomes with functional centromers and big rearranged chromosomes.

Various forms of aberrant mitoses, discovered in mouse G1 cell line, testify to the functional disorders of both chromosomes proper and the whole mitotic apparatus [4, 15]. The increased chromosomal instability and intensive course of the karyotypic evolution of cells of the investigated cell line, along with mitotic pathologies, point out the damage of mitosis regulation, *i.e.* the loss of the mitotic checkpoint, or the spindle checkpoint. Misregulation of the normal course of cell cycle results in disbalancing of reaction cascades of all cell processes that they depend on.

Last but not the least is the fact that DNA repair systems of G1 cells were also damaged, which is evidenced in various chromosomal aberrations (breaks, in particular). The euploid cells and aneuploids of different groups of ploidy, as well as chromosome aberrations, including RT, create the variety of genetically different clones and, therefore, provide the material for the selection of the most adaptable cell clones to the conditions of the culture. And such cells are the transformed cells with the damaged genome stability regulation.

The question arises – do the analogous changes of chromosomal apparatus appear at cultivation of human stem cells, suggested for therapeutic usage? As even keeping up to the basic conditions of stem cells cultivation, in mouse G7 cell line, genome mutations are observed also [3], moreover, there are data of accumulation of the mutations in embryonic stem cell populations during cultivation [16].

Conclusions. Intensive karyotypic evolution, accompanied by increased chromosomal instability, and the long-term establishment from the moment of cell line stabilization on the 140th pasage are specific to mouse G1 cell line. The domination of high ploid cells (near-pentaploids, in particular) and the significant percentage of cells with RT on all the stages of establishment of the investigated cell line were revealed.

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