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The Sources of Heterogeneity of Embryo Cell Populations of Mouse Line BALB/c Cultured *In Vitro*

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Detailed chromosomal analysis of embryo mouse cell line G1 on the 15th passage of in vitro cultivation has been performed. Possible mechanisms of heterogeneity of the investigated cell line are discussed. Spontaneous chromosome G-staining has been observed in some metaphase plates. Premature disjunction of sister centromeres has been found, in comparison with other regions of sister chromatids. The distribution of rearranged chromosomes (including Robertsonian translocations), the presence of autonomous chromosome clusters, associated in pericentromeric regions, as well as despiralization of pericentromeric heterochromatin blocks designate the similarity of metaphase chromosomes of a novel mouse cell line G1 to embryonic stem cells and embryonic germ cells of rodents.

Keywords: mouse cell line, cell population heterogeneity, chromosomal aberrations.

Introduction. The study on pluripotent stem cells of embryonic origin occupy the central position among the investigations on molecular mechanisms of cell differentiation, development of cell therapy, as well as in obtaining transgenic mammals, the main source of which is the internal cell mass of blastocysts and primordial cells of germs of generative organs. The main problem of using stem cells is connected with the unsuccessful attempts to develop the methods and to discover the identification features which would allow foreseeing the direction of their development *in vitro*, dynamics of pluripotence loss, and the restrictions on their ability to form the generative line cells during obtaining chimeric animals at their participation. Therefore, stable cell lines, obtained from embryonic germinal cells (EGC) are of special significant.

It is noteworthy that the number of known mouse and human embryonic stem cell (ESC) lines, which are being studied at different laboratories of the world, reaches several dozens, while there are only a few lines of EGC stable cell lines at the moment, in particular only single human EGC lines have been obtained [1, 2].

According to the data in [3], one of the tissue-specific features of germinal cells of murine rodents in perinatal period is the splitting of sister centromeric regions of chromosomes, preceding the splitting of corresponding cromatids. There are some experimental evidences of a certain contribution of tissue-specific patterns of chromosomal DNA methylation in these cells, i.e. hypomethylation of centromeres and hypermethylation of other regions [4-6].

Our previous studies on mouse ESC of R1 line revealed metaphase plates in which preceding splitting of sister centromeric regions in comparison to other areas of sister chromosomes has been observed. The evident heterogeneity of cell subpopulations on the

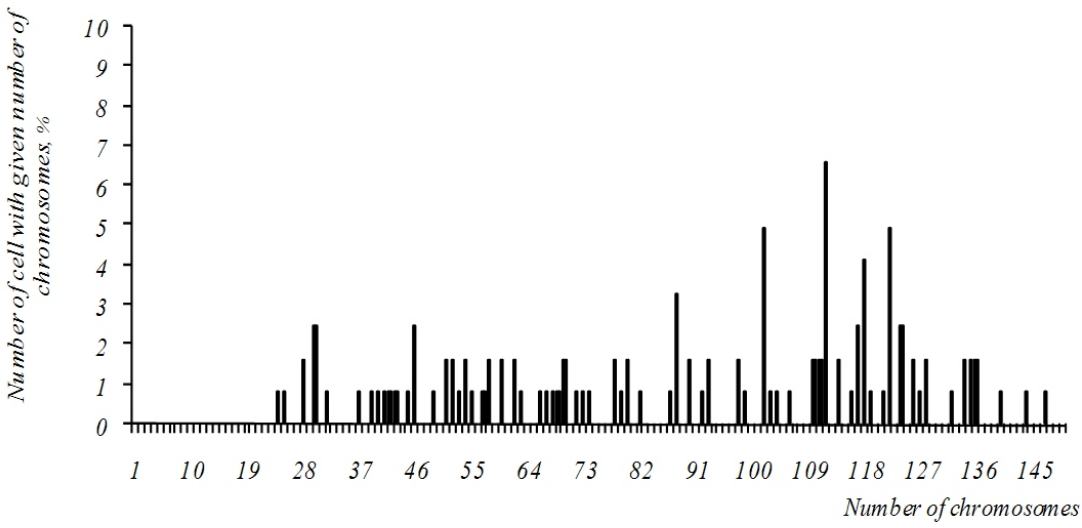

Fig.1. Distribution of G1 line cells by the number of chromosomes at the 15th passage

basis of this phenomenon has been discovered, regardless of their common origin and beyond dependences on different passages after ESC isolation out of the blastocyst internal cell mass of mouse cell line 129 [7, 8]. To evaluate this phenomenon in embryonic cell populations of different origin, including EGC populations, and its possible influence on karyotypic evolution of cells at their passaging *in vitro,* the analysis of metaphase plates, obtained from the 15th passage of G1 cell line, previously described by us [9], has been performed in the present work.

Material and Methods. Initially, the cells, obtained from genital eminence of 12.5-day old mouse embryo of BALB/c line have been used [9]. The method of metaphase plates obtaining is presented in details in [10]. Stained cytogenetic preparations were analyzed using binocular microscope "Carl Zeiss" (Austria), 1000x magnification. The captures of metaphase plates were made by Canon "Power Shot G6" (Canon, UK).

Results and Discussion. The data obtained show that G1 cell line populations at the 15th passage are characterized by a wide variety of cells with different number of chromosomes (Fig.1). The issue of the polyploids appearance turned out to be rather complicated. 1 to 7 Robertsonian translocations (RT) or centric fusions between acrocentric chromosomes are observed in polyploid cells. In some hyperdiploid cells, the number of chromosomes in which varied from 57 to 120 (i.e. they were close to tri-, tetra-, penta-, and hexaploids) simultaneous presence of odd number of RT has been revealed, Fig.2 presents the examples of them. Owing to the fact that some spontaneous differential staining of chromosomes (G-bending) occurred in some metaphase plates, we were able to typify individual chromosomes. The presence of two copies of homologous RT, e.g. Rb (12; 18), was detected, in other cells the same but rearranged chromosome RT (12; 18) in a single copy and some additional RT of different origin in a single copy were detected as well. The data obtained suggest that in the investigated cell populations polyploidization takes place at least in two possible ways: i) the omission of cytokinesis, due to which the marker chromosomes should be duplicated and ii) intercellular fusion, as a result of which in cells one copy of each marker chromosome from different cell clones is present.

Noteworthy is the fact that in the mouse ESC of R1 line, studied by us earlier [7, 8] as well as in the cell populations of G1 line at the 15th passage the metaphases with spontaneous G-bending have been observed. For instance, Fig.3 shows such metaphase, the majority of chromosomes of which were typified. It contains 121 chromosomes (including arms of three

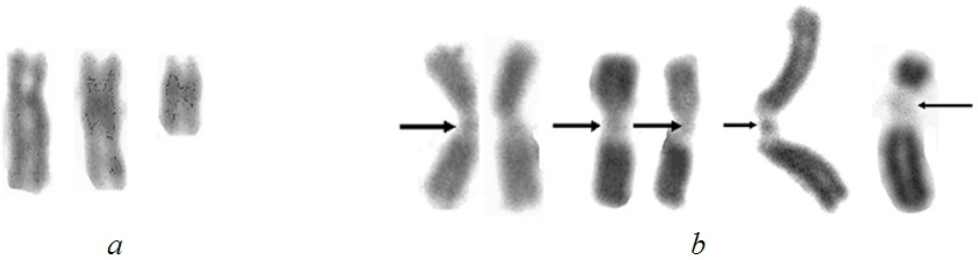

Fig.2. Despiralization of pericentrometric heterochromatin and preceding splitting of sister centromeres in different metaphase plates of the 15th passage of G1 line cells. a – acrocentric chromosomes of mouse; b – Robertsonian translocations (heterochromatin blocks in the sites of fusion are indicated by arrows)

RT), 28 of which are rearranged. Not rearranged autosomes are generally presented by 5-6 copies, except for the largest ones, such as chromosomes 1-7, the number of copies of which varied from 1 to 4 (Fig.3). One more common feature of metaphase chromosomes of ESC R1 line and EGC R1 line at 15th passage is that in some metaphase plates the preceding splitting of sister centromeres was observed in comparison with other areas of sister chromatids (Fig.2, a). It is typical for perinatal generative cells of murine rodents [3]. Fig.2 shows that in the majority of chromosomes of different metaphase plates the connection between sister chromatids is preserved due to the presence of heterochromatin blocks along sister chromatids, as it was earlier described [3]. Despiralization of centromeric regions is detected at the absence of large pericentromeric heterochromatin blocks typical for centromeric regions of the mouse chromosomes. Thus, two chromosomes 5 were revealed in the same metaphase plate, different in presence/absence of pericentromeric heterochromatin block and centromeric splitting. Despiralization of pericentromeric heterochromatin is also clearly seen in the series of RT in Fig. 2, b. Moreover, the compacted blocks (indicated by arrows in Fig.2) are observed in the despiralized region, which joints two chromosomes into RT.

Despiralization of centromeres is observed in metaphase chromosomes in EGC G1 line preparations at the 15th passage, regardless of the fact that in order to accumulate metaphases the cells were treated by colchicine, which, according to [3], diminishes the frequency of preceding splitting of sister centromeres in EGC significantly. In accordance with the same authors [3], such despiralization of centromeres and their early splitting is accompanied by a significant increase in centromeric fusions and complicated chromosomal rearrangements, which have been discovered in our investigations as well. It seems like this very fact conditions all known restrictions in ESC and EGC filling of generative organs at the obtaining of chimeric mice with their usage [11].

The necessity of physical contact between the chromosomes is an obligatory condition for the formation of interchromosomal rearrangements. RT are considered to be formed in the chromocenters of interphase nucleus, where the centromeric regions of acrocentric chromosomes are closely located. The residues of such interchromosomal associations along pericentromeric heterochromatin blocks are also observed in metaphase plates of mouse BALB/c line bone marrow cells, which are obtained without any colchicine. Similar associations are revealed in the cell populations of EGC G1 line, which restrict the centromeric fusions and interchromosomal exchange (Fig.4). Two focuses of interchromosomal associations, where interchromatide centromeric fusions take place, have been revealed in 11% of investigated hyperdiploid cells.

Therefore, the data obtained allow us to make the following conclusions. The EGC populations of G1 line at the 15th passage are characterized by high heterogeneity in both the number of chromosomes and the presence of the rearranged chromosomes, RT in particular. The distribution of the rearranged chromosomes along hyperploid cells suggests that the certain contribution to the heterogeneity observed is made not by hyperploidization only (by means of omitting the stage of cytokinesis) but due to the intercellular fusions as well.

One of the features of chromosomal apparatus instability is the presence of autonomous clusters of chromosomes in the metaphase plates, which are

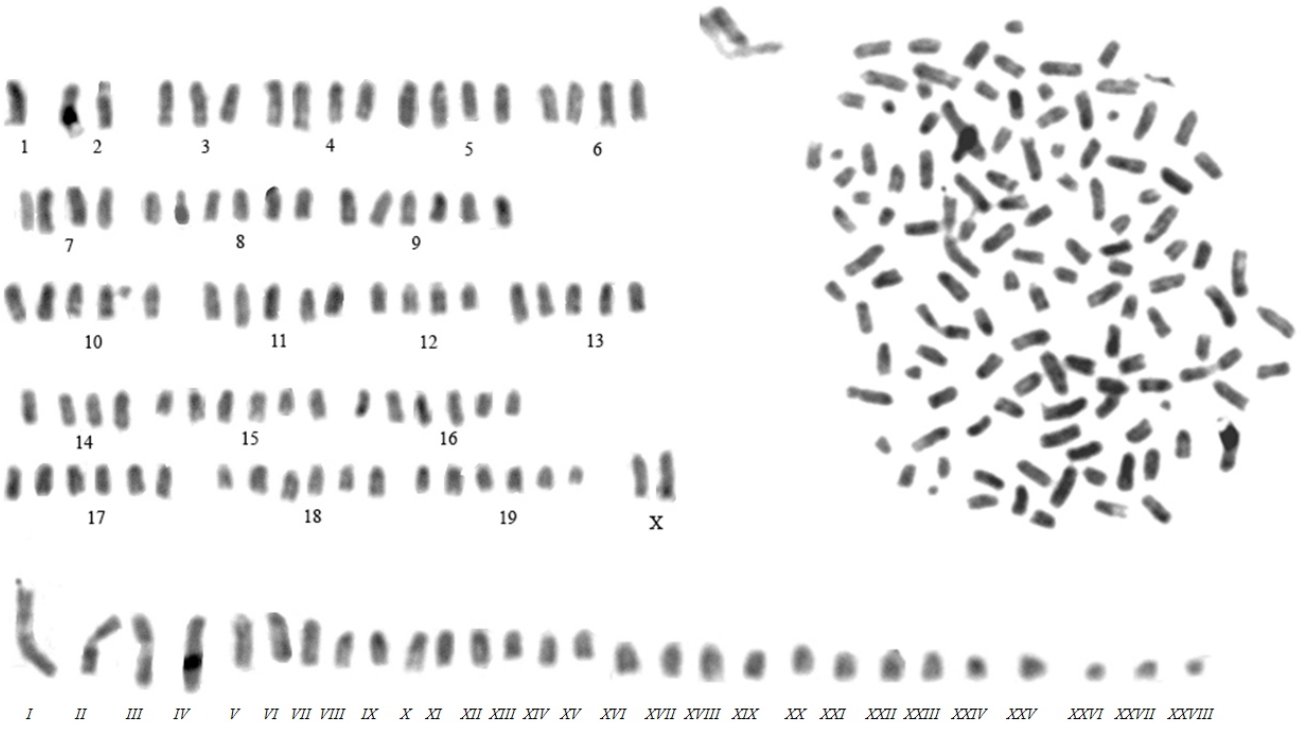

Fig.3. Spontaneous differential staining of metaphase chromosomes of the 15th passage of G1 line cells (three Robertsonian translocations, 121 arms of chromosomes, Arabic numerals indicate not rearranged chromosomes, Roman numerals indicate the rearranged ones)

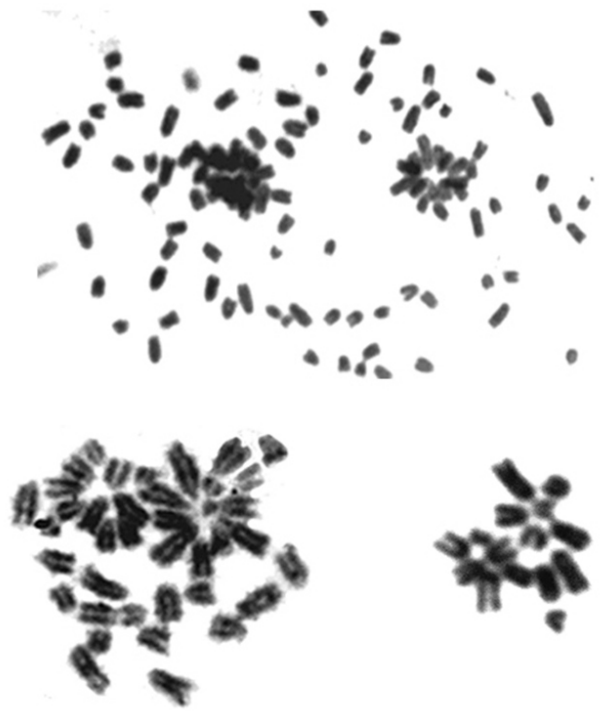

Fig.4. Clusterization of chromosomes, associated in centromeric regions into two autonomous groups in the metaphase plates of the 15th passage of G1 line cells.

associated by the pericentromeric regions, which, perhaps, reflects the predisposititon to the formation of multipolar mitosis. One more source of intrapopulational changeability, which is clearly distinguished in this kind of cells, is the despiralization of pericentromeric heterochromatin blocks, promoting the formation of RT as well as complicated interchromosomal exchanges, clearly revealed in the groups of chromosomes forming clusters in the centromeric regions. The data testifying to the metaphase presence with spontaneous G-bending in cell populations of G1 line at the 15th passage correlate with the results of ESC investigations of mouse R1, and on the basis of chromosomes with despiralized pericentromeric chromatin they correlate with the typical morphology of perinatal cells of generative series, described for murine rodents.

As a conclusion it should be noted that just the tissue-specific despiralization of pericentromeric heterochromatin, accompanying the formation of complicated chromosomal defects results in the restriction in filling of generative organs by the

progenies of embryonic stem cells of different cell lines (ESC, EGC) during obtaining chimeric animals with their usage.

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Источники гетерогенности культивируемых *in vitro* популяций эмбриональных клеток мыши линии BALB/c

Резюме

Выполнен детальный хромосомный анализ клеток линии G1 мыши эмбрионального происхождения на 15-м пассаже культивирования in vitro. Обсуждаются возможные механизмы гетерогенности исследуемой клеточной линии. В некоторых метафазных пластинках наблюдали спонтанное дифференциальное окрашивание хромосом, а также обнаружили опережающее расщепление сестринских центромер по сравнению с остальными участками сестринских хроматид. Распределение перестроенных хромосом, в том числе и робертсоновских транслокаций, наличие автономных кластеров хромосом, ассоциированных в перицентромерных участках, и деспирализация перицентромерных гетерохроматиновых блоков указывают на сходство метафазных хромосом новой клеточной линии G1 мыши с эмбриональными стволовыми клетками и эмбриональными герминативными клетками мышевидных грызунов.

Ключевые слова: стволовые, эмбриональные, герминативные клетки, кариотипическая гетерогенность.

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