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SPONTANEOUS PREMATURE CONDENSATION OF CHROMOSOMES IN NORMAL AND TRANSFORMED MAMMAL CELLS

*O.A. Kovaleva[1], T.T. Glazko[2], T.P. Kochubey[2], L.L. Lukash[2], Yu.I. Kudryavets[1], **

[1]*R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kiev, Ukraine*

[2]*Institute of Molecular Biology and Genetics NAS of Ukraine, Kiev, Ukraine*

***Aim*: To study the relation between premature chromosome condensation and the ability of the cells to undergo malignant transformation. *Methods*: Standard cytogenetic analysis of bone marrow cells and cultured normal and tumor cells has been used. *Results*: Comparative analysis of the frequency of occurrence of the cells with premature chromosome condensation (PCC) (cell "arrest" at G2/M phase) in relation to dividing cells in the cultures of human immortalized cells of hematopoietic origin, human lung carcinoma A-549 cells, and in populations of bone marrow cells of BALB/c and C57BL/6 mice differing in predisposition for myeloma development has been performed. It has been revealed that in populations of bone marrow cells of C57BL/6 mice the relation of cells with PCC to dividing ones is 2–3-fold lower than in other studied cell populations. Immortalized and malignantly transformed human cell lines were characterized by high frequency of occurrence of cells with PCC. In the cells of A-549R subline characterized by suppressed malignant phenotype this index was lower than in parental A-549 cells. *Conclusion*: The obtained data point on possible relation between disturbed passing of "check point" by cells upon transition from G2 phase of cell cycle to mitosis and increased genetic heterogeneity of new cell generation associated with ability of cells to immortalization and malignant transformation.**

***Key Words:* chromosomes, mitotic catastrophe, normal and transformed cells.**

It is known that in mammals chromosome condensation is tightly related to dissociation of nuclei envelope at early prophase of mitosis. However, in some cases such condensation is occurring before beginning of mitosis and this phenomenon was termed as "premature chromosome condensation" (PCC). Firstly it was observed more than 30 years ago in the nuclei of interphase cells during their fusion with cells at the phase of mitosis upon the action of Senday virus. Approximately 10 years ago the method of exogeneous induction of PCC has been developed. Whislt PCC, induced by fusion was provided by migration of maturation/mitosis promoting factor — MPF — from mitotic cells to interphase ones, exogeneous induction of PCC was initiated by addition of inhibitors of proteinphosphatases (for example, caliculine A), able to activate intracellular MPF, to culture medium [1]. It was shown that elevation of the frequency of occurrence of interphase cells with PCC is closely related to cell death [2]. It is supposed that PCC could be a precursos of one of the variants of cell death – "mitotic catastrophe" (MC), that occurs with the involvement of intracellular mechanisms that are different from these upon apoptosis [3, 4]. MC could be observed after the action of a number of stress factors, including heat shock, chemical agents, irradiation, and is characterized by altered cell morphology [5]. PCC is an early stage of this process. Later such cells do not reach cytokinesis, or divide and nearly immediately after that fuse together forming the figures typical for the death by MC type. At the same time it has been shown that the part of the cells survives MC, and in their next generations the high frequency of occurrence of aneuploids and polyploids could be detected [5, 6]. Some researchers are supposing that the high frequency of occurrence of spontaneous PCC is typical for tumor cells: it is associated with defective "check point" at the G2/M stage of cell cycle (disfunction of TP53 protein, accumulation of cycline B1, activation of cdc2-cycline dependent kinase) and serves as a sourse of genetic heterogeneity, immortalization and adaptation of cell populations to influence of anticancer drugs and irradiation [4–7].

To understand whether the phenomenon of spontaneous "premature chromosome condensation" is typical for malignant transformation, in the present reseach we have performed comparative analysis of the frequency of occurence of such cells in malignantly transformed or immortalized human cell lines as well as in bone marrow cells of mice of lines BALB/c and C57BL/6, differing in their predisposition to develop tumors. The BALB/c line (but not C57BL/6 line) is characterized by genetically determined predisposition to develop myeloma (www.jak.org).

MATERIALS AND METHODS

In the study, cell populations of human cell lines (lung adenocarcinoma A-549 cell line and its cell subline A-549R adapted to high concentration of interferon), immortalized human cell lines obtained from peripheral blood of the donor (fibroblast-like 4BLFibroline and its subline 4BLC21) were analyzed. To obtain cell suspension, the cells were incubated

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*Correspondence: Fax: 038 (044) 258-16-56
E-mail: kudrya@onconet.kiev.ua

Abbreviations used: MC – mitotic catastrophe; MPF – maturation/mitosis promoting factor; PCC – premature chromosome condensation.

in 0.25% tripsin/versen solution for 10 min at +37 °C. Then cells were centrifuged at 400 g for 5 min, cell pellet was resuspended in 0.56% KCl and incubated for 30 min at +37 °C. Then the cells were triply fixed in solution of methanol/acetic acid (3 : 1).

BALB/c and C57BL/6 mice were obtained from the vivarium of the Institute of Molecular Biology and Genetics NAS of Ukraine (Kiev, Ukraine). The work with the animals has been approved by Local Ethic Committee. The samples of bone marrow cells were standardly prepared (without use of colchicine): bone marrow was washed from femoral bone by 0.56% KCl and incubated in this solution at 37 °C for 20 min, then fixed with the mixture of methanol/acetic acid (3 : 1). Then all fixed cell suspensions were plced by drops on cold wet glass slides, dryied and stained by Heymza stain (Merck, Germany). These preparations were examined using binocular Axiostar Plus microscope (Carl Zeiss, Germany) at the magnification X1000. Metaphase plates were photographed using camera Canon PowerShot G5 (UK). The frequency of occurrence of interphase cells with PCC, dividing cells, binuclear cells, cells with micronuclei and apoptotic cells were counted per 1000 cells, and the results are expressed in per mille (‰). Statistical difference was evaluated using Student's *t*-criterium.

RESULTS AND DISCUSSION

The nuclei with premature chromosome condensation are shown on Fig. 1, 2. The combination of the characteristics of interphase nuclei (the presence of nuclear envelope and nucleoli) and metaphase plate (the presence of typical metaphase chromosomes, in some cases dividing in telomeres regions to chromatids, Fig. 2, *a*) is evidencing that the cells are in transition from G2 phase of cell cycle to mitosis. The count of cells with such nuclei has shown that there is certain tendency between the frequency of their occurrence and the number of dividing cells, as well as the frequency of occurrence of apoptotic cells, but not the cells with micronuclei (Table 1). At the same time, some authors are supposing that apoptosis is at some degree alternative to PCC and following cell death by MC [4]. It is considered that entry of cells to apoptosis is determined at the period of G1/S phase, and the cells that reach G2 phase with defects at

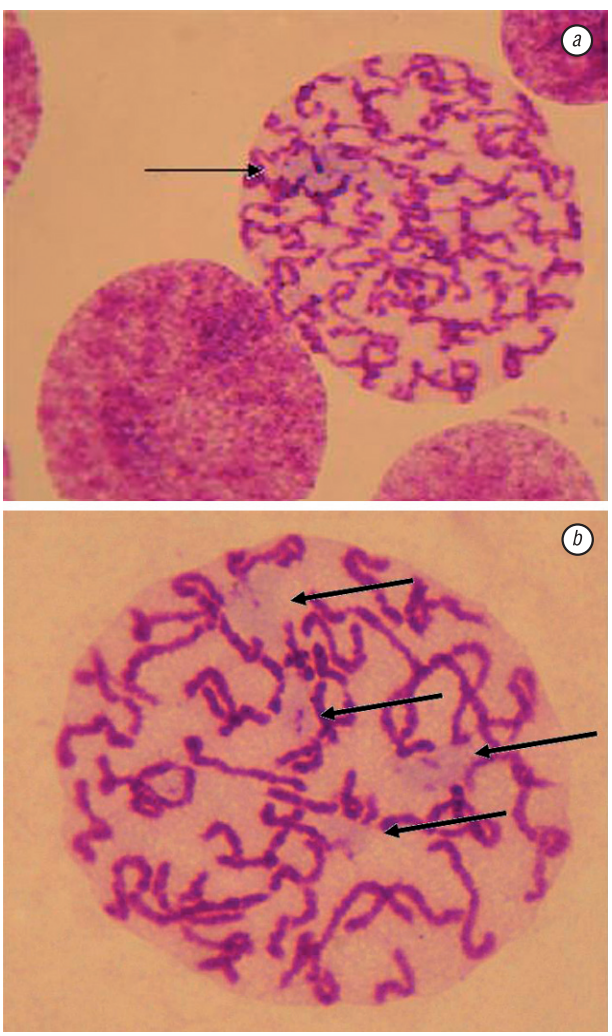

Fig. 1. "Premature chromosome condensation" in human lung carcinoma cells of A549 line (*a*) and its subline A549R (*b*). Arrows point on nucleoli

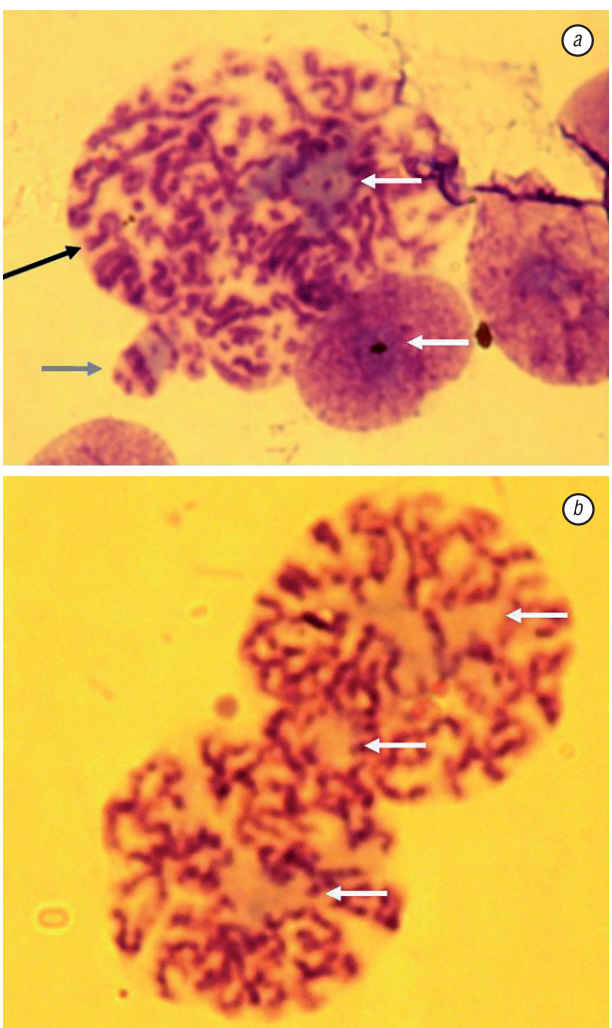

Fig. 2. "Premature chromosome condensation" in the 4BLFibro cells. *a* – mononuclear cell; *b* – binuclear cell. An example of chromosome divided to chromatides is pointed by black arrow. Nucleoli are pointed by white arrows. The fragment of nucleoli tightly contacting with the condensed chromosomes is pointed by gray arrow

"check point" at G2/M period and arrest at mitosis, have two ways: or death by MC, or arrest at G2 phase with the next polyploidy and restoration of ability to cell division [4].

Table 1. The frequency of occurrence of cell divisions, cells with micronuclei, premature chromosome condensation, and apoptotic cells in A-549 cell line and A-549R subline

Cell line (subline)	Frequency of occurrence (‰)				
	Interphase nuclei with PCC	% in relation to dividing cells	Dividing cells	Cells with micronuclei	Apoptosis
A-549 (8000 cells)	5.6 ± 1.0	50	12.0 ± 1.6	2.5 ± 0.5	4.0 ± 1.4
A-549R (7000 cells)	1.6 ± 0.7	46	3.5 ± 0.5	5.8 ± 0.6	1.2 ± 0.6

The relation between the cells with PCC (arrested at G2/M period) and total pool of dividing cells (metaphase, anaphase) was evaluated. It has been revealed that for cells of A-549 and A-549R lines the frequency of occurrence of such cells is > 50% in relation to dviding cells (Table 1), i.e. nearly 1/3 cells that reached G2/M stage of cell cycle was arrested at this stage.

The relation between the number of cells with PCC and the rate of cell divisions has been found also in the populations of immortalized cells of 4BLFibro and 4BLC21 lines (Table 2). The decreased number of dividing cells was accompanied in some cases by appearance of metaphases, where the presence of nucleoli was clearly observed (Fig. 3). It is known that dissociation of nuclear envelope, release of nuclueolar protein nucleophosmine, is a key event in arrest of duplication of centrosomes and prevention of multipolar mitosis [8]. One could expect that the disturbance of synchrony between dissociation of nuclear envelope, chromosome condensation and involution of nucleoli could serve as additional indicator of disturbance of cell division and predisposition to elevated genetic variability in the next generations of the cells.

Table 2. The frequency of occurrence of cell divisions, and cells with premature chromosome condensation in cell lines 4BLFibro and 4BLC21

Cell line	Frequency of occurrence (‰)				
	Metaphase	Interphase nuclei with PCC	% in relation to dividing cells	Dividing cells	Metaphase with nucleoli
4BLFibro	0.15	0.54	78	0.69	0
4BLC21	0.09	0.75	73	1.03	0.19

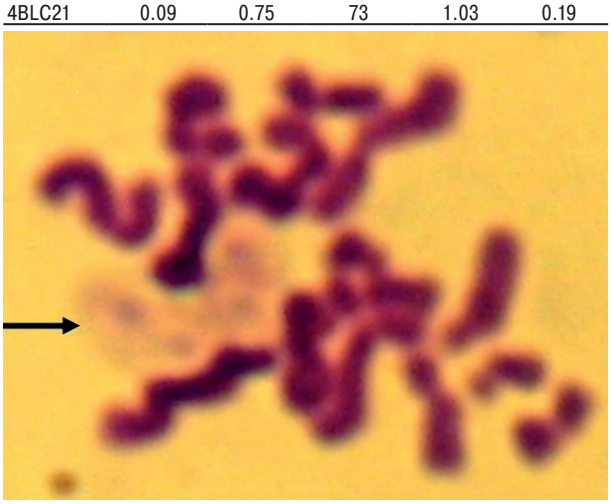

Fig. 3. Fragment of metaphase plate of the 4BLFibro cell with a nucleus-like structure (pointed by arrow)

So, mentioned above human cell lines were characterized by high frequency of occurrence of the cells arrested at G2/M stage in relation to the pool of dividing cells. It is necessary to note that the frequency of occurrence of PCC in the cells of A-549R subline characterized by decreased malignancy patterns due to prolonged exposition to interferon, is significantly lower than that in parental A-549 cells (Table 1). Interestingly, the number of cells with PCC, detected by us in different human cell lines, is close to that reported for immortalized hamster ovarian cells (5–15‰) [2].

To answer the question if such phenomenon is typical exactly to cell cultures *in vitro* and is associated with ability of cells to acquire malignant patterns, we have performed comparative study of the frequency of occurence of PCC in the populations of bone marrow cells of BALB/c and C57BL/6 mice, differing in predisposition to myeloma development.

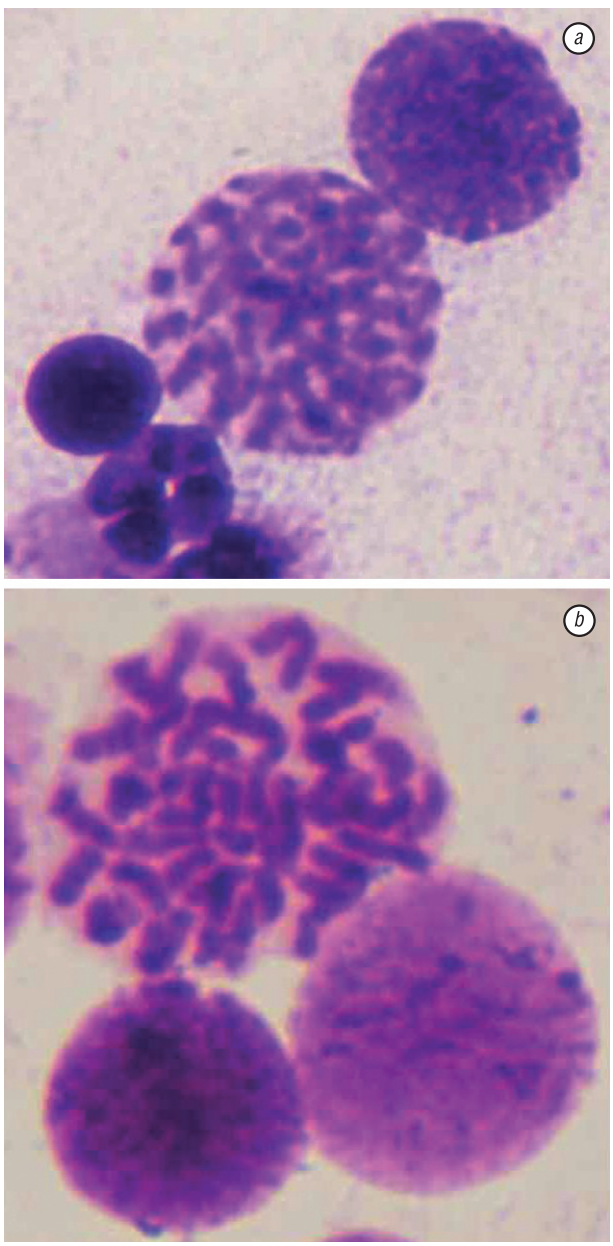

Fig. 4. "Premature chromosome condensation" in bone marrow cells of BALB/c mice (*a*) and C57BL/6 mice (*b*)

In bone marrow cells from both murine strains, the cells with PCC were detected (Fig. 4, Table 3). It was shown that the groups of mice of one line as well as between the lines differ significantly by the frequency of cell divisions. However, in both groups of BALB/c mice the ratio of the cells with PCC to the total pool of dividing cells is similar to that found in human tumor cell lines (Table 3), i.e. more than 1/3 part of the cells that reached mitosis, is arrested at G2/M stage of cell cycle. But in bone marrow cells of C57BL/6 this index is not exceeding 22% (Table 3), being 2–3 times lower than such indexes in other studied cell populations (Tables 1–3).

Table 3. The frequency of occurrence (‰) of interphase nuclei with premature chromosome condensation, dividing cells, binuclear cells and polyploids in bone marrow cells of mice

Bone marrow cells	Frequency of occurrence (‰)			
	Interphase nuclei with PCC	% in relation to dividing cells	Dividing cells	Binuclear cells
BALB/c	3.9 ± 1.2	76	5.1 ± 1.4	5.6 ± 1.5
C57Bl/6	0.7 ± 0.3	13	5.5 ± 1.3	3.7 ± 0.9

So, the obtained data are supporting the hypothesis that elevated frequency of occurence of the cells with PCC could be associated with the one of the mechanisms of generation of genetic heterogeneity in the populations of the cells predisposed to malignant transformation [4–7]: such disturbance of cell cycle as altered cynchrony of chromosome condensation, dissociation of nuclear envelope and involution of nucleoli may lead to increased number of aneu- and polyploidy cells the appearance of which may promote acquirement of malignant patterns.

In the study, we did not reveal the direct relation between elevated frequency of occurence of the cells with PCC and such cytogenetic abnormalities like elevated number of cells with micronuclei, binuclear cells (Tables 1, 3). At the same time it is known that despite the fact that the part of the cells undergoing arrest at G2/M stage of cell cycle and restoring clonogenic ability is very low [9], phenomenon of PCC may lead to the appearance of genetically heterogenous populations, new clones with increased resistance to environmental influences [4, 6].

We have observed that in part the cells with PCC may die by apoptosis: there wer dying cells which morphology resembled the intermediate variant between chromatin condensation typical for apoptosis (Fig. 5, *a, b*) and distribution of condensed chromatin between separate chromosomes characteristic for PCC (Fig. 5, *c*). From this point of view one should note that elevated frequency of occurrence of PCC in A-549 cells compared to A-549R subline is associated also with elevated rate of apoptosis (Table 1).

In conclusion our data allow to propose that the ratio of the cells with premature chromosome condensation (the disturbance of synchrony between dissociation of nuclear envelope, chromosome condensation and involution of nucleoli) to the total pool of dividing cells may be used as an additional cytogenetic characteristic for prognosis of genetic instability of cell populations.

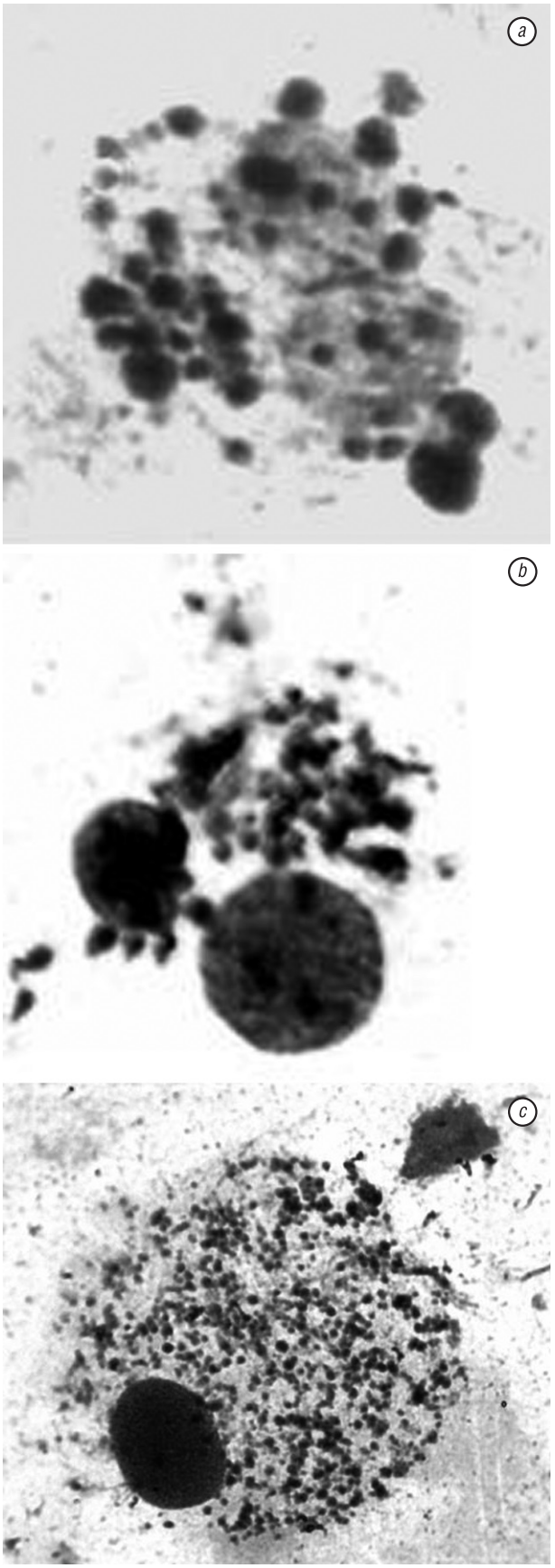

Fig. 5. Apoptosis in bone marrow cells of BALB/c mice (*a, b*) and human A549 cells (*c*)

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СПОНТАННАЯ ОПЕРЕЖАЮЩАЯ МИТОЗ КОНДЕНСАЦИЯ ХРОМОСОМ В НОРМАЛЬНЫХ И ТРАНСФОРМИРОВАННЫХ КЛЕТКАХ МЛЕКОПИТАЮЩИХ

***Цель*: изучение взаимосвязи между преждевременной конденсацией хромосом и склонностью клеток к злокачественной трансформации. *Методы*: стандартные методы цитогенетического анализа клеток костного мозга и культивируемых нормальных и опухолевых клеток. *Результаты*: проведен сравнительный анализ отношения частоты выявления клеток с преждевременной конденсацией хромосом (PCC) (арест клеток в G2/M) к делящимся клеткам в культурах иммортализованных клеток человека кроветворного происхождения и злокачественно трансформированных клеток рака легкого человека линии A-549, а также в популяциях клеток костного мозга двух линий мышей: линии BALB/c с высокой предрасположенностью к развитию миеломы и C57BL/6, без такой предрасположенности. Выявлено, что в популяциях клеток костного мозга мышей низкораковой линии C57BL/6 отношение клеток с PCC к делящимся в 2–3 раза меньше, чем в других исследованных клеточных популяциях. Иммортализованные и злокачественно трансформированные клеточные линии человека характеризовались высокой частотой присутствия клеток с PCC. В клетках сублинии A-549R, характеризующейся подавлением признаков злокачественности, этот показатель заметно ниже, чем в клетках исходной линии A-549. *Выводы*: полученные данные позволяют предположить связь между нарушением прохождения клетками точки проверки при переходе от G2 фазы клеточного цикла к митозу и повышенной генетической гетерогенностью их потомства, ассоциированной со склонностью клеток к иммортализации и злокачественной трансформации.**

***Ключевые слова*: хромосомы, митотическая катастрофа, нормальные и трансформированные клетки.**