ISSN 0233–7657. Biopolymers and Cell. 2013. Vol. 29. N 6. P. 480–486 doi: 10.7124/bc.00083D

UDC 576.32; 575.113; 577.15

Correlation of mutagenesis level with expression of reparative enzyme O^6-methylguanine DNA methyltransferase during establishment of cell lines *in vitro*

L. L. Macewicz[1], V. O. Kushniruk[1], A. P. Iatsyshyna[1], K. V. Kotsarenko[1], V. V. Lylo[1], G. R. Akopyan[2], N. L. Huleuk[2], D. O. Mykytenko[3], L. L. Lukash[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03680

[2]Institute of Hereditary Pathology, NAMS of Ukraine
31a, Lysenka Str., Lviv, Ukraine, 79000

[3]Clinic of reproductive medicine «Nadiya»
19A, M. Krivonosa Str., Kyiv, Ukraine, 03037

l2m@gazeta.pl

Long-term cultivation of cell lines inevitably leads to genetic and epigenetic changes. ***Aim.*** *A comparative analysis of karyotype and level of the expression of reparative enzyme O^6-methylguanine-DNA-methyltransferase (MGMT) at different stages of establishment and stabilization of human cell line 4BL and cell line of mouse germ cells G1.* ***Methods****. The set of methods was used to research the dynamics of karyotypes changes: the differential staining of chromosomes, FISH-method and comparative genomic hybridization. The level of MGMT expression was analyzed by PCR reaction and Western blot analysis.* ***Results****. General trends of establishment of mouse and human cell lines were revealed: at the first stage, which is characterized by increased structural instability of the genome, an increase in the MGMT expression was revealed while at the second stage of stabilization – a decrease in the expression. Therefore, almost complete disappearance of MGMT protein in unmodified form (24 kDa) is observed.* ***Conclusions****. Statistically significant correlation between MGMT repair enzyme and mutations induction processes during mammalian cell adaptation and cell line establishment to in vitro was described.*

Keywords: cell line, karyotypic evolution, O^6-methylguanine-DNA-methyltransferase (MGMT), genomic instability.

Introduction. Cell lines of different origin are widely used in many laboratories all over the world, beginning with fundamental research in science and ending with biotechnological synthesis in industry and cellular therapy [1, 2]. Nearly 4 000 cell lines are maintained in cell bank of ATCC. Majority of human permanent cell lines are of cancer origin, malignant and characterized by considerable karyotypes changes, disruption of mitotic spindle and aberrant regulation of mitotic apparatus [3, 4]. Most of human permanent cell lines were either obtained from tissues of embryonic origin, or immortalized by viral transformation or by introducing vector designs with specific genes, for instance, telomerase gene [5, 6]. Only about 30 cell lines are normal, but they are primary cultures. Therefore, the novel cell line 4BL, obtained from peripheral blood of healthy donors in our department, is of particular interest. The cell line 4BL successfully passes Hayflick limit and is cultivated for more than 220 passages.

We also derived novel mouse embryonic germ cells lines G1, G4, G6 and G7 from genital bumps of 12.5-day embryos of laboratory mouse of inbred line BALB/c.

An adaptation of cells to artificial support *in vitro* and lack of immune system inevitably lead to the change in primary characteristics of cell, including the karyotypic one. The clones of cells with selective advantages are characterized by higher genetic instability [3]. They tend to give rise to the cell line due to their better survival rate, and act as a source of further genetic and epige- netic changes [3, 7].

DNA methylation is a major mechanism of epigenetic regulation of genes [8, 9]. Undesirable DNA methylation occurs upon the action of endogenous and exogenous alkylation compounds. Methylation of O^6 position of guanine is especially dangerous, as O^6-methylguanine is recognized as adenine, so it binds with thymine instead of cytosine [10]. As a result, the transitions GC > AT are generated [11]. Reparative enzyme O^6-alkylguanine-alkyltransferase (AGT), better known as O^6-methylguanine-DNA-methyltransferase (MGMT), repairs this type of damage, transferring methyl residue to its own cysteine residue [12]. We have investigated the expression of MGMT at different stages of the development of the human and mice cell lines to test the activity of reparative system in response to mutational processes that occur during the adaptation of cells to cultivation conditions *in vitro*. The cytogenetic analysis was performed to determine the nature of chromosomal rearrangements at different stages of the formation and stabilization of cell lines, and also after the treatment of cells with stress factors. Thus, the aim of the research was a comparative analysis of the karyotypes and the level of expression of MGMT at different stages of formation and stabilization of the human cell line 4BL and the line of embryonic germ cells of mouse G1.

Materials and methods. We used the cell population of the new cell lines 4BL obtained from the peripheral blood of a healthy donor [13] and a new line of embryonic germ cells of mouse G1 derived from genital bumps of 12.5-day embryos of laboratory mouse inbred line BALB/c [14]. The cells were cultivated in standard medium DMEM («Sigma», USA) with the addition of 100 U/ml penicillin, 100 μg/ml of streptomycin and 10 % fetal calf serum.

Chromosome preparations for cytogenetic studies were obtained according to the standard protocol [6, 15]. Metaphase chromosomes were stained by 10 % of Giemsa stain, GTG-staining and differential QFH/AcD-coloring. Chromosomes were analyzed at the magnification of 1000 times, using a light microscope Olympus BX41, equipped with automated analysis system «CytoVision 4.01». 200 metaphase plates (m. p.) of each passage were examined. The chromosomes structure was analyzed at the level of 400–550 bands per haploid set. To determine the structural rearrangements, the international cytogenetic nomenclature ISCN-2013 was used [16].

For Western-blot analysis, the proteins were isolated from the samples and concentration was measured by Bradford's method [17]. The protein extract was studied using SDS-electrophoresis in 12 % of polyacrylamide gel by Lemmli [18]. We used monoclonal antibodies against MGMT («Novus Biologicals Littleton», USA) and secondary species-specific antibody conjugated with horseradish peroxidase («Jackson ImmunoResearch», USA). Identification of MGMT in cells extract was conducted according to the recommendation of the antibodies manufacturer. Control of the uniformity of protein application was carried out by densitometric evaluation of the total amount of protein transferred to the membrane, by the program Origin Pro 8.5.

Establishment of mouse germ cell line G1. To identify common patterns of genomic evolution during the establishment of cell line G1, the standard cytogenetic analysis was conducted at 24, 53, 68, 104 and 140 passages. The distribution of frequency of individual karyotypes is presented in Fig. 1.

The normal mouse karyotype includes 19 pairs of autosomes and one pair of sex chromosomes, so diploid set of chromosomes $2n = 40$. At 24^{th} passage slight divergence of distribution curves was observed with peaks in near-diploid and near-hexaploid area. By 53 passages the frequency of near-diploid cells decreased, and by 68 passage distribution pattern changed dramatically: the distribution curve became asymmetric, with a peak in near-diploid region and subsequent almost linear decrease of frequency of karyotypes with high number of cells. However, at 104 passage the frequency of near-diploid cells dropped sharply, modal class of near-hexaploid cells was detected. At 140 passage near-hexaploid modal class remained, a small peak in near-tetraploid area and a trace pool of near-diploid cells were observed.

It is known, that the Hayflick limit for most primary cultures of human and mouse cells is 30–50 divisions

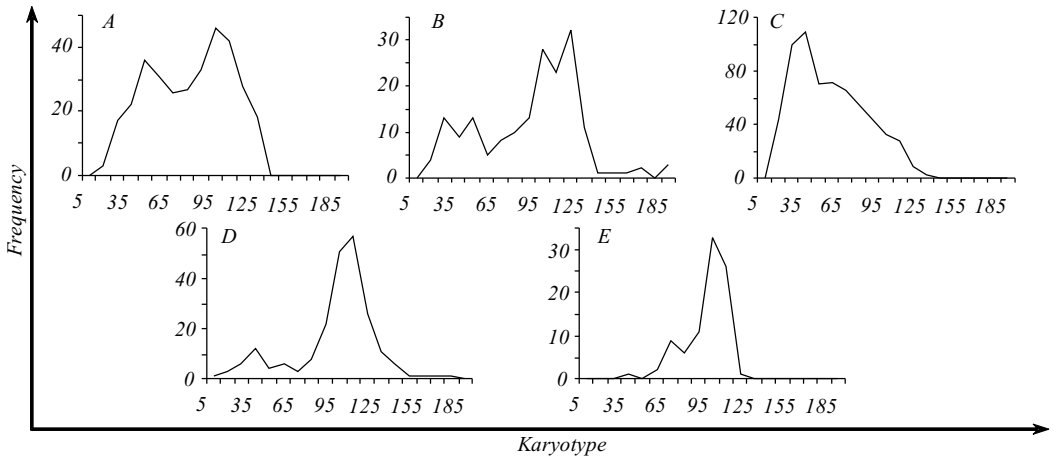

Fig. 1. Distribution of karyotypes frequencies at different stages of karyotypic evolution of mouse cell line G1. Passages: *A* – 24; *B* – 53; *C* – 68; *D* – 104; *E* – 140

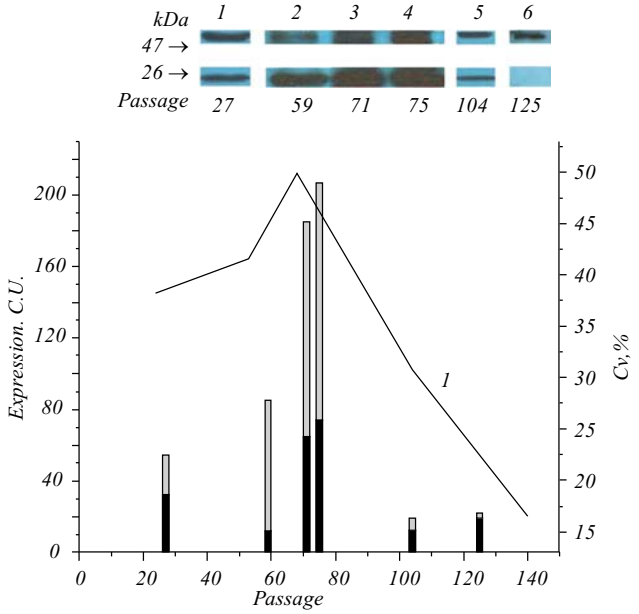

Fig. 2. Expression of reparative enzyme MGMT at different stages of the establishment of mouse cell line G1(Western blot analysis) (*A*) and genetic instability and level of the expression of reparative enzyme MGMT at different stages of the establishment of mouse cell line G1 (the coefficient of variation of karyotypes frequencies and densitometric analysis of western blot hybridization of MGMT expression) (*B*:*grey* – 23 kDa; *black* – 50 kDa; *1* – cv, %)

[19]. Reaching this limit is accompanied by the aging of cell culture, and the onset of the stage of terminal stop of proliferation. Probably, in case of cell line G1, the high-ploid cells reached the Hayflick limit earlier, so in cell population, close to 68 passage, the selection took place in the direction of near-diploid cells. During the immortalization of cell line, the aging stage is changing to the stage of crisis [20]. The majority of cells die, but a small percent of cells survive and become capable of unlimited division due to the karyotype rearrangements, changes in gene dosage and aberrant regulation of the mitotic apparatus.

Thus, dramatic change in the distribution of frequency of individual karyotypes and pivotal change in the direction of karyotypic evolution during short period of time from 53 to 68 passages are most probably explained by the crisis of cell line G1, and its transition to the immortalized state.

During further development of the cell line G1, there was no stabilization of near-diploid karyotype, and the trends to polyploidization were observed: most of the cell population had a near-hexaploid karyotype to 140th passage. As shown previously [21], this phenomenon is due to the weakening of mitosis checkpoint and mutation of one allele of the gene Trp53, which leads to the functional inactivation of p53.

This protein is involved in regulation as a regulator of a number of cellular processes, including – DNA repair [22]. The expression of the reparative enzyme MGMT is indirectly regulated with the involvement of p53. It has been shown, that p53-deficient cells have a reduced baseline of MGMT [23].

Analysis of the MGMT expression at the protein level in the cell line G1 was performed (Fig. 2, *A*). At early stages of the evolution of cell line a steady increase in the MGMT expression was detected. The maximum value of this index was fixed at 71 and 75 passages – that is, at approximately the same stage of the cell line, at which a sharp change in direction of karyotypic evolution was observed.

We revealed that the functional inactivation of p53 did not cause the deficit of MGMT. We have sugges-

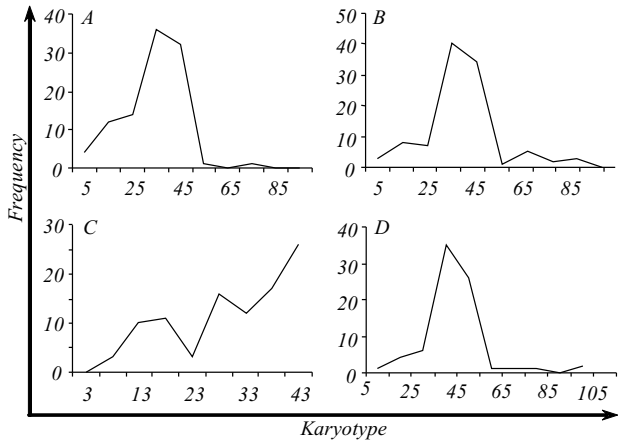

Fig. 3. Distribution of karyotype frequencies at different stages of karyotypic evolution of human cell line 4BL. Passages: *A* – 70; *B* – 122; *C* – 133; *D* – 206

ted that this phenomenon was associated with the presence of a powerful factor that causes the induction of MGMT – namely, genetic material lesions [24], which accompanied the establishment of cell line G1. These injuries were manifested in the form of cells polyploidization and were accompanied by numerous structural rearrangements, including a significant percentage of Robertsonian translocations.

As an indicator of genetic instability of the culture was chosen the coefficient of variation, calculated for distribution of karyotypes frequencies at different stages of the cell line establishment. The comparison of these data with the data of Western blot analysis is shown in Fig. 2, *B*. Both analyzed parameters increased at the early passages, reached maximum values at 70–80 passages, and declined at the later (< 100) passages.

Since the data obtained did not allow direct calculation of the correlation coefficient between these two parameters, an interpolation of the curves was performed, using software OriginPro8.1. The data were also used to calculate the Pearson correlation coefficient.

The calculated value of the correlation coefficient for unmodified form of MGMT was 0.41, with a significance level $p \leq 0.05$ – whereas the reliable correlation for this enzyme in modified form was not found.

Interestingly, that the dynamics of frequencies of Robertsonian translocations had distinct inverse correlation with the coefficient of variation of the distribution of frequencies of karyotypes. It was maximal at the stabilization stage of the cell line. This is probably due to the fact that the formation of Robertsonian translocation is the mechanism of reduction of the number of chromosomes in the process of polyploidization.

Thus, two main stages of the evolution of the cell line G1 are described:

– establishment stage, accompanied by increasing structural instability and induction of reparative enzyme MGMT;

– stabilization stage, characterized by the formation of near-hexaploid modal class, and lower levels of the expression of MGMT protein.

Establishment of human cell line 4BL. The same research was carried out on material of cell populations of the line 4BL. Analysis of the distribution of karyotypes frequencies at different stages of karyotypic evolution of the cell line is presented in Fig. 3.

In contrast to the mouse germ cell line G1, polyploidization was not typical for karyotypic evolution of the cell line 4BL: near-diploid modal class remained at all stages of karyotypic evolution. At 133 passages the nature of distribution changed: a curve of disturbance became asymmetric with maximum in near-diploid area, almost complete lack of hyperploid, and pronounced peak in the area of 11–20 chromosomes were observed. Thus, we can assume that this stage of the cell evolution was a crisis stage and cell line became immortalized, then the stabilization stage followed.

Similarly to the mouse germ cell line G1, the establishment stage of karyotypic evolution of the cell line 4BL was also accompanied by the increase of structural instability, then came the stabilization phase.

The comparative data on the coefficient of variation of karyotypes frequencies and the level of the expression of MGMT protein at different stages of cultivation of the cell line 4BL are presented on the Fig. 4. The Pearson correlation coefficient was calculated, it was 0.59 ($p \leq 0.001$).

It should be noted that, as opposed to the mouse cell line G1, the MGMT protein in unmodified form completely disappeared at the stabilization phase of the cell line 4BL. To exclude the possibility of a complete deletion of the gene MGMT, the comparative genomic hybridization was conducted at the 205 passage of cultivation of the cell line 4BL. The presence of *MGMT* promoter and its methylation status were studied by PCR analysis.

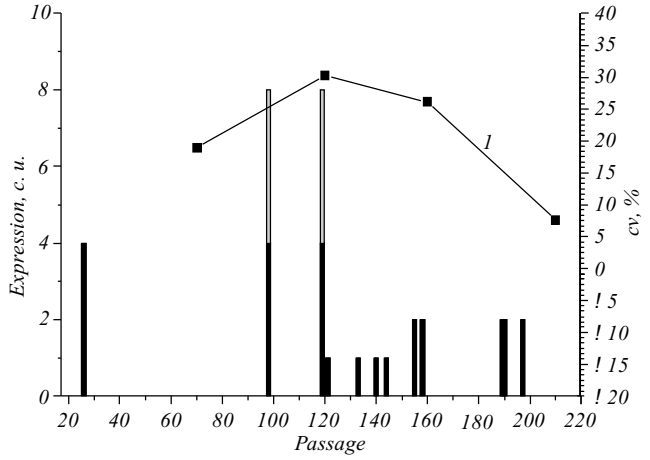

Fig. 4. Genetic instability and level of the expression of reparative enzyme MGMT at different stages of the establishment of human cell line 4BL (the coefficient of variation of karyotypes frequencies and densitometric analysis of western blot hybridization of MGMT expression): *grey* – 23 kDa; *black* – 50 kDa; *1* – cv, %

The results of comparative genomic hybridization indicate the presence in the cell population of deletion in 10q26 region, where the *MGMT* gene is located, but this deletion has mosaic character, confirming the results of PCR analysis. Thus, the total loss of this gene in the cell line 4BL does not occur at any stage of its karyotypic evolution.

The study on the promoter methylation level demonstrates that in the cell population 4BL both methylated and unmethylated alleles were revealed (the research was performed by Iatsyshyna in the laboratory of B. Kaine). So, it cannot be postulated that the MGMT protein in unmodified form (24 kDa) disappears due to the lack of transcription of this gene.

We can conclude that although the nature of chromosomal and genomic mutations in the cell lines G1 and 4BL differ significantly, the stages of structural instability and stabilization had different duration, but in both lines there was observed a correlation between the level of structural genomic instability and the level of expression of reparative enzyme MGMT.

Perhaps this is one of the compensatory mechanisms activated by the genome destabilization and the stress factors, associated with the adaptation of the cells to cultivation *in vitro*. This assumption is confirmed by the data of [12], which showed the key role of MGMT in the survival of the cell population under stress condition and under the influence of mutagens with different mechanisms of the damaging effects.

To verify the above-mentioned assumption, the cell line 4BL, which was on the early phase of stabilization (passage 165) was subjected to the stress of destabilizing factor: cultivation for 24 h in medium M2, which has altered ionic composition, and is characterized by increased ionic strength. Karyotyping was performed at the 160 passage (before stress factor action) and at the 205 passage. Nullisomy or monosomy of 10^{th} and 13^{th} chromosomes and monosomy of chromosomes 4, 8, 11, 15, 17, 21, *X* were observed; six regular marker chromosomes were revealed. The most representative were such aberrations: t (1, 11) (q12; p15) – 63 % of m. p., del (2) (p11 ~ 12) – 46 % m. p., t (5; 15) (p10; q10) – 23 % m. p., t (12, 15) (p10; q10) – 10 % m. p. and t (16; 21) (q13; p11) – 5 % m. p. Modal class karyotype at 160^{th} and 205^{th} passages was 42–43 chromosomes. Percentage of polyploid cells increased from 2.8 to 36 %.

Changes in the coefficient of variation of karyotypic evolution are presented in Fig. 5. From 160^{th} passage the coefficient of variation for this characteristic noticeably decreased, but after exposure to stress factors, sharp rise of the curve with the formation of a new peak, higher than the previous one, was observed. According to the results of cytogenetic analysis, the cells processing with the stress factor probably led to the reconversion of the cell population to the state of structural instability.

At the same time, the analysis of MGMT expression in the cell lines, that were exposed to the same stress factor (Fig. 6) was performed. Immediately after treatment, clear inhibition of the MGMT expression was observed with the almost complete disappearance of the protein, but within 10 days after treatment this phenomenon changed to induction of the expression of MGMT protein in modified form, this pattern persisted through the next 5 days, and in 26 days the appearance of enzyme in unmodified form was revealed.

Based on our and literature data presented above, we can assume that complete inhibition of expression of MGMT in both modified and unmodified forms under the influence of the medium M2 with high ionic strength led to the disruption of cells adaptation to cultivation in vitro that, in turn, caused the re-emergence of structural genomic instability. On the other hand, the increased genome structural instability led not only to increasing the expression of MGMT protein in modified

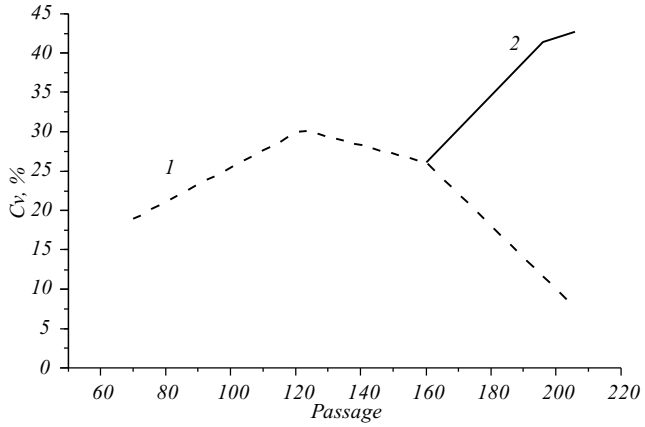

Fig. 5. Induction of genetic instability in cell line 4BL by medium with high ionic strength M2: *1* – intact; *2* – treated by M2

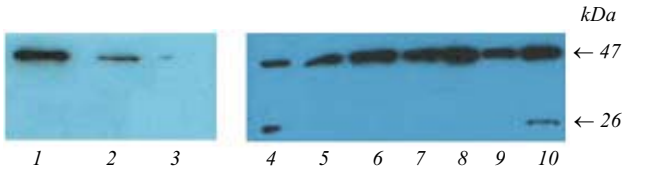

Fig. 6. Cnange of the expression of reparative enzyme MGMT in control cells and in cells, treated with medium M2: *1* – control; *2* – cells cultivation with M2; *3* – next passage after incubation with M2; *4* – positive control (cell line U937); *5* – control to variant 6; *6* – 10 days after treatment; *7* – control to variant 8; *8* – 15 days after treatment; *9* – control to variant 10; *10* – 26 days after treatment with M2 medium

form, typical for the cell line 4BL at the stabilization stage, but also to the induction of the expression of MGMT in unmodified form, which was typical for the cell line 4BL at the establishment stage, characterized by high structural instability of the genome.

Conclusions. The adaptation of cells to cultivation *in vitro* is accompanied by the induced mutation process associated with the period of structural genomic instability. The immortalization of cell lines is accompanied by the crisis stage, which is characterized by the sharp increasing of genomic instability and intensive selection in the cell populations. During further evolution of the cell lines, the stage of structural instability is changed by the stabilization stage, with the formation of dominant modal class with a certain number of chromosomes.

Statistically significant correlation of the MGMT expression with the degree of genomic instability during spontaneous immortalization and establishment of the cell lines was revealed.

Complete inhibition of the MGMT expression under the stress factor on the stage of cell line stabilization led to the re-growth of genomic instability in the cell populations, indicating the essential role of this enzyme in the maintenance of adaptation of the established cell lines to cultivation *in vitro*. The re-growth of genomic instability after exposure to the stress factor, in turn, led to the increased expression of MGMT, in particular, to the induction of expression of this protein in its canonical (24 kDa) form.

Л. Л. Мацевич, В. О. Кушнірук, А. П. Яцишина, К. В. Коцаренко, В. В. Лило, Г. Р. Акопян, Н. Л. Гулеюк, Д. О. Микитенко, Л. Л. Лукаш

Кореляція змін рівня мутагенезу і експресії репаративного ферменту O^6-метилгуанін-ДНК метилтрансферази за становлення клітинних ліній *in vitro*

Резюме

Тривале культивування клітинних ліній неминуче призводить до виникнення генетичних та епігенетичних змін. ***Мета****.Порівняльний аналіз каріотипу і рівня експресії репаративного ферменту O^6-метилгуанін-ДНК метилтрансферази (MGMT) на різних етапах становлення та стабілізації клітинної лінії людини 4BL і лінії ембріональних гермінативних клітин миші G1.* ***Методи****. Зміни каріотипу в динаміці досліджували, використовуючи диференційне забарвлення хромосом, FISH-метод і порівняльну геномну гібридизацію. Ген MGMT та його експресію вивчали за допомогою ПЛР та Вестерн-блот аналізу.* ***Результати****. Період становлення ліній клітин миші та ліній клітин людини має загальні тенденції: на першому етапі, який характеризується підвищеною структурною нестабільністю геному, виявлено зростання рівня експресії гена MGMT, а на другому – етапі стабілізації – зниження рівня експресії даного гена. При цьому спостерігали практично повну відсутність немодифікованої форми білка MGMT (24 кДа).* ***Висновки****. Встановлено достовірну позитивну кореляцію рівня експресії репаративного ферменту MGMT з мутаційними процесами, які виникають при адаптації клітин до умов культивування у період становлення ліній клітин ссавців.*

Ключові слова: клітинна лінія, каріотипічна еволюція, O^6-метилгуанін-ДНК метилтрансфераза (MGMT), геномна нестабільність.

Л. Л. Мацевич, В. О. Кушнирук, А. П. Яцишина, К. В. Коцаренко, В. В. Лыло, Г. Р. Акопян, Н. Л. Гулеюк, Д. А. Микитенко, Л. Л. Лукаш

Корреляция изменений уровня мутагенеза и экспрессии репаративного фермента O^6-метилгуанин-ДНК метилтрансферазы при становлении клеточных линий *in vitro*

Резюме

Длительное культивирование клеточных линий неизбежно приводит к возникновению генетических и эпигенетических изменений. ***Цель****. Сравнительный анализ кариотипов и уровня экспрессии репаративного фермента MGMT на разных стадиях становления и стабилизации клеточной линии человека 4BL и линии эмбриональных герминативных клеток мыши G1.* ***Методы****. Исследовали изменение кариотипа в динамике с использованием дифференциальной окраски хромосом, FISH-метода и сравнительной геномной гибридизации. Ген MGMT и его экспрессию изучали с помощью ПЛР и Вестерн-блот-анализа.* ***Результаты****. Период становления клеточных линий человека и мыши характеризуется общими тен-*

денциями: на первом этапе, где наблюдается повышенная структурная нестабильность генома, выявлено возрастание уровня экспрессии гена MGMT, а на втором – этапе стабилизации – снижение уровня экспрессии указанного гена. При этом отмечено практически полное отсутствие немодифицированной формы белка MGMT (24 кДа). ***Выводы****. Обнаружена достоверная позитивная корреляция уровня экспрессии репаративного фермента MGMT с мутационными процессами, возникающими при адаптации клеток к условиям культивирования в период становления клеточных линий млекопитающих.*

Ключевые слова: клеточная линия, кариотипическая эволюция, O^6-метилгуанин-ДНК метилтрансфераза (MGMT), геномная нестабильность.

REFERENCES

1. *Russell C., Rahman A., Mohammed A. R.* Application of genomics, proteomics and metabolomics in drug discovery, development and clinic // Ther. Deliv.–2013.–**4**, N 3.–P. 395–413.
2. *Sng J., Lufkin T.* Emerging stem cell therapies: treatment, safety, and biology // Stem Cells Int.–2012.–**2012**.–P. 521343.
3. *Gagos S., Iliopoulos D., Tseleni-Balafouta S., Agapitos M., Antachopoulos C., Kostakis A., Karayannakos P., Skalkeas G.* Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: studies on two immortal colon cancer cell lines // Cancer Genet. Cytogenet.–1996.–**90**, N 2.–P. 157–165.
4. *Thompson S. L., Bakhoum S. F., Compton D. A.* Mechanisms of chromosomal unstability // Curr. Biol.–2010.–**20**, N 6.–R285–295.
5. *Kingham E., Oreffo R. O.* Embryonic and induced pluripotent stem cells: understanding, creating, and exploiting the nano-niche for regenerative medicine // ACS Nano.–2013.–**7**, N 3.–P. 1867–1881.
6. *Freshney R. I.* Culture of animal cells: a manal of basic technique and specialized applications / Ed. R. Ian Freshney.–New Jersey: Wiley, 2010.–796 p.
7. *Kanatsu-Shinohara M., Ogonuki N., Iwano T., Lee J., Kazuki Y., Inoue K., Miki H., Takehashi M., Toyokuni S., Shinkai Y., Oshimura M., Ishino F., Ogura A., Shinohara T.* Genetic and epigenetic properties of mouse male germline stem cells during long-term culture // Development.–2005.–**132**, N 18.–P. 4155–4163.
8. *Mueller W. C., von Deimling A.* Gene regulation by methylation // Recent Results Cancer Res.–2009.–**171**.–P. 217–239.
9. *Huidobro C., Fernandez A. F., Fraga M. F.* The role of genetics in the establishment and maintenance of the epigenome // Cell Mol. Life Sci.–2013.–**70**, N 9.–P. 1543–1573.
10. *Fu D., Calvo J. A., Samson L. D.* Balancing repair and tolerance of DNA damage caused by alkylating agents // Nat. Rev. Cancer.–2012.–**12**, N 2.– P. 104–120.
11. *Halford S., Rowan A., Sawyer E., Talbot I., Tomlinson I.* O^6-methylguanine methyltransferase in colorectal cancers: detection of mutations, loss of expression, and weak association with G:C > A:T transitions // Gut.–2005.–**54**, N 6.–P. 797–802.
12. *Kaina B., Christmann M., Naumann S., Roos W. P.* MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents // DNA Repair (Amst).–2007.–**6**, N 8.–P. 1079–1099.
13. *Lukash L. L., Iatsyshyna A. P., Kushniruk V. O., Pidpala O. V.* Reprogramming of adult somatic human cells *in vitro* // Factors of experimental evolution.–Kyiv: Logos, 2011.–Vol. 11.–P. 493–498.
14. *Lukash L. L., Iatsyshyna A. P., Pidpala O. V., Vagina I. N., Kochubey T. P.* Obtaining of new mouse stem cell line and investigation the microenvironment impact on karyotypic evolution // Physiology and Biochem. of Cultivated Plants.–2006.–**38**, N 2.–P. 144–152.
15. *The AGT* cytogenetics labolatory manual / Eds M. J. Barch, T. Knutsen, J. L. Spurbeck.–Philadelphia: Lippincott-Raven, 1997.–688 p.
16. *An* International System for Human Cytogenetic Nomenclature / L. G. Shaffer, J. McGowan-Jordan, M. Schmid.–Basel: Karger publ., 2013.–140 p.
17. *Bradford M. M.* A rapid and sensitive method for the quantitation of microgram quantities of protein-dye binding // Anal. Biochim.–1976.–**72**.–P. 248–254.
18. *Laemmli U. K.* Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature.–1970.–**227**, N 5259.–P. 680–685.
19. *Hayflick L., Moorhead P. S.* The serial cultivation of human diploid cell strains // Exp. Cell Res.–1961.–**25**.–P. 585–621.
20. *De Silva R., Whitaker N. J., Rosan E. M., Reddel R. R. HPV-16 E6* and *E7* genes, like SV40 early region genes, are insufficient for immortalization of human mesothelial and bronchial epithelial cells // Exp. Cell Res.–1994.–**213**, N 2.–P. 418–427.
21. *Iatsyshuna A. P., Kvasha S. M., Pidpala O. V., Ruban T. P., Vagina I. M., Lukash L. L.* Genetic instability of mouse embryonic germ cell line G1 and misfunction of mitotic check-point and p53 // Biopolym. Cell.–2007.–**23**, N 4.–P. 338–346.
22. *Albrechtsen N., Dornreiter I., Grosse F., Kim E., Wiesmuller L., Deppert W.* Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53 // Oncogene.–1999.–**18**, N 53.–P. 7706–7717.
23. *Grombacher T., Eichhorn U., Kaina B.* p53 is involved in regulation of the DNA repair gene O^6-methylguanine-DNA methyltransferase (MGMT) by DNA damaging agents // Oncogene.–1998.–**17**, N 7.–P. 845–851.
24. *Fritz G., Kaina B.* Stress factors affecting expression of O^6-methylguanine-DNA methyltransferase mRNA in rat hepatoma cells // Biochim. Biophys. Acta.–1992.–**1171**, N 1.–P. 35–40.

Received 01.04.13