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SHORT COMMUNICATIONS

The expression of O^6-methylguanine-DNA methyltransferase in the spontaneously immortalized mouse cell line G1 and its sublines G1-OA and G1-T

A. P. Iatsyshyna, V. V. Lylo, O. V. Pidpala, T. P. Ruban, I. M. Vagina, L. L. Lukash

The Institute of Molecular Biology and Genetics
150 Zabolotny Str., Kyiv 03143, Ukraine

a.p.iatsyshyna@imbg.org.ua

The expression of Mgmt gene was investigated by Western blot analysis in the spontaneously immortalized mouse cell line G1 and in its sublines G1-OA and G1-T at different passages of in vitro cultivation. The highest level of Mgmt expression has been revealed in G1-T subline cells and the lowest – in G1-OA subline cells. The increase in the level of DNA repair enzyme Mgmt was observed in the cells of mouse cell line G1 as well as in its subline G1-OA at later passages of in vitro cultivation. Since the G1-OA subline is characterized by the highest frequencies of chromosomal aberrations, micronucleate and multinucleate cells, it is possible to suppose a role of deficiency of DNA repair enzyme Mgmt in the increase of the chromosomal aberration level in G1-OA subline.

Key words: mammalian cells in vitro, O^6-methylguanine-DNA methyltransferase (Mgmt), chromosomal aberrations

Introduction. O^6-methylguanine-DNA methyltransferase (also O^6-alkylguanine-DNA alkyltransferase, in human gene *MGMT*, in mice – *Mgmt*) is one of the enzymes of DNA repair, which protects the cell from mutagenic effect of of alkylating carcinogens [1, 2]. MGMT enzyme functions by a stoichiometric reaction of self-inactivation, the mechanism of which involves the transfer of alkyl group (mostly methyl group) from O^6-position of guanine (O^6MeG) of DNA molecule to cysteine in active site of enzyme [1]. *Mgmt* gene is expressed in both normal and malignant cells, but the expression level varies depending on the type of cells and their growth characteristics [3]. Thus, *MGMT* was reported to have higher expression level in some neoplasia cells, in particular, resistant to alkylating compounds or to reduce of *MGMT* expression at malignant transformation [3-6].

The reactions of methylation and ethylation of guanine in O^6 position are considered to be the main carcinogenic factors, regardless of the minority of such DNA damages [7]. If the alkylated bases are not repaired with MGMT before the next replication, then these damages are realised in gene mutations (transitions from guanine:cytosine to adenine:thymine), sister chromatid exchanges, chromosomal aberration, death or malignant transformation of cells [7]. It has been demonstrated that during the MGMT deficiency or its functional inactivation, O^6MeG is involved to the formation of secondary DNA damages, which include ab-

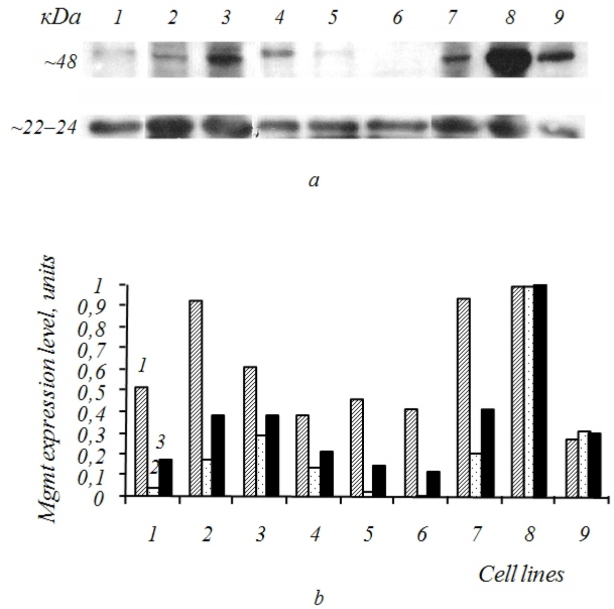

Fig.1 Analysis of Mgmt gene expression in cell lines: 1 – G1, 59^{th} passage; 2 – G1, 71^{st}; 3 – G1, 75^{th}; 4 – G1-OA, $35/26^{th}$; 5 – G1-OA, $35/40^{th}$; 6 – G1-OA, $35/44^{th}$; 7 – 3T3; 8 – G1-T, $66/13^{th}$; 9 – MCF-7 (a – Western blot analysis of MGMT protein; b – diagram demonstrating the relative level of MGMT protein: 1 – 22-25 kDa; 2 – 48 kDa; 3 – total amount of the protein). Cell extracts (250 µg of total protein per hole) were sepa-

errations of chromosomes [7-10], although the mechanism of such damages has not been studied enough.

The results of cytogenetic analysis of spontaneously immortalized mouse cell line G1 [11] and its sublines show the differences in numbers of various chromosomal aberrations.

The goal of this work was to analyse the expression of *Mgmt* at the level of protein in the cells of new G1 line and its sublines, G1-OA and G1-T, at different passages of *in vitro* cultivation and to reveal the correlation between aberrations of chromosomes and the levels of the investigated DNA repair protein.

Materials and Methods. The mouse cell line G1 was obtained from material of embryonic origin of BALB/c mouse strain at the Department of Human Genetics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine; the sublines G1-OA and G1-T were selected by the criteria of transformed phenotype *in vitro* [12, 13]. 12.5 days post coitum (dpc) embryos of BALB/c mice were used for obtaining the G1 cell line [12]. The cell lines of mice 3T3 (embryo) [14] and human MCF-7 (adenocarcinome of mammary gland), which was selected as positive control, both obtained from Saint Petersburg collection of cell lines [15] were used.

MGMT level was determined by Western blot analysis using monoclonal anti-MGMT antibodies (*Novus Biologicals*, USA). Species-specific antibodies conjugated with horseradish peroxidase (*Jackson ImmunoResearch*, USA) were used as secondary antibodies. Protein extracts were obtained in accordance with recommendations [16]. Concentration of total protein in cell lysates was determined using the Bradford's quantitative method [17]. Gel-electrophoresis of protein extracts and Western blot analysis were carried out according to recommendations [16, 18]. X-ray films were scanned using Ultrascan XL densitometer (*LKB*, Sweden).

Results and Discussion. The application of Western blot analysis of the invetigated cells with antibodies to MGMT allowed defining a series of proteins with molecular weight of at ~22-24 and ~48 kDa (Fig.1, 2), which is a specific reaction of monoclonal antibodies, as the similar signals have not been observed after the treatment of membrane with 1%-solution of bovine serum albumin instead of primary antibodies with subsequent manipulations in accordan with the manufacturer's protocol. The dependence of MGMT protein detection on the type of ready protiens storing has been marked out in the range of ~22-24 and ~48 kDa, as well as the dependence from the variety of cell culture (the data is not presented). Therefore, the data from both ranges ~22-24 and ~48 kDa have been included into the analysis of detected MGMT protein. Detected protein (molecular weight 48 kDa) may be considered to be the product of either alternative splicing of mRNA or reconstructed gene, or protein complex, which requires further detailed investigation.

Fig.1 shows that the level of *Mgmt* expression in mouse G1 cell line at the 59^{th} passage of *in vitro* cultivation was lower than at the 71^{st} and 75^{th} passages. The total amount of MGMT protein at the 71^{st} and 75^{th} passages did not differ essentially. The amount of MGMT protein in lysates of G1-OA subline cells at $35/26^{th}$, $35/40^{th}$, and $35/44^{th}$ passages of cultivation was lower than the one of the original G1 line at the 70^{ths} passages. The decrease in total amount of

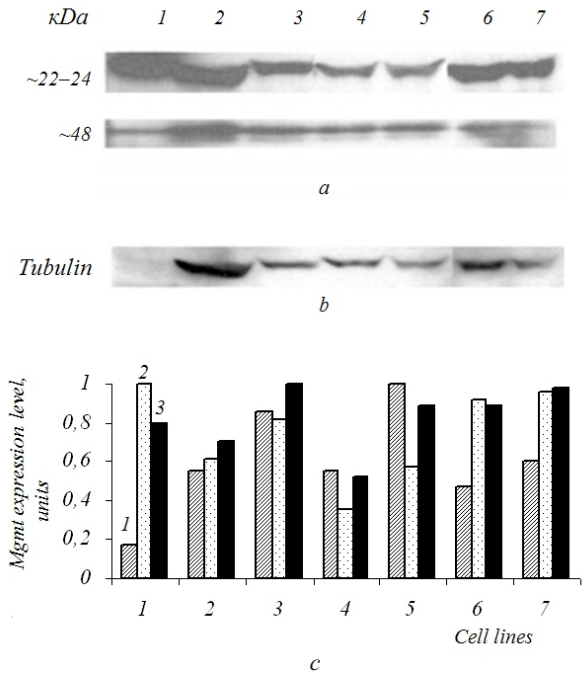

Fig.2 Analysis of Mgmt gene expression: 1 – BALB/c embryo; 2 – G1, 27th passage; 3 – G1, 104th; 4 – G1-OA, 35/24th; 5 – G1-OA, 35/65th; 6 – G1-T, 66/17th; 7 – G1-T, 66/35th (a – immunodetection of MGMT protein in the areas of ~22-24 kDa using anti-MGMT antibodies; b – Western blot analysis using anti-tubulin antibodies (~55 kDa), which was served as loading control; c – diagram, demonstrating the relative MGMT protein level: 1 – 22-24 kDa; 2 – 48 kDa; 3 – total protein amount). Cell extracts (100 μg of total protein per hole) were separated in 15%-polyacrilamide gel by SDS-electrophoresis.

the investigated protein with the increased passages of G1-OA cells has been observed. The highest level of MGMT protein was detected at 66/13th passage. MGMT protein was detected in cells of 3T3 mouse cell line, which is conditionally normal cell line with balanced karyotype.

The levels of MGMT protein were compared in lysates of BALB/c mouse embryo cells, in original G1 cell line at the 27th and 104th passages, in cells of its sublines G1-OA at the 35/24th and 35/65th passages and G1-T at 66/17th and 66/35th passages of *in vitro* cultivation (Fig.2).

The area of ~22-24 kDa is specific for the lowest level of MGMT protein in BALB/c embryonic cells (Fig.2). The cells of original G1 line and its sublines G1-OA and G1-T demonstrated general tendency towards the increase in amount of MGMT protein in cells at the later passages of *in vitro* cultivation. The area of ~48 kDa revealed specific signals of MGMT protein and the same pattern of increase of amount of DNA repair protein at later passages of cell cultures (Fig.2). Additional signals of specific immune reaction were detected in BALB/c embryonic cells, except for the areas of ~22-24 and ~48 kDa (data are not presented). Summarizing the results of the analysis of total amount of MGMT protein in the investigated cultures, it is worth mentioning the significant increase in the amount of MGMT in G1 cells at the 104th passage, comparing to the 27th passage, as well as in the cells of G1-OA subline at late 35/65th passage of cultivation, comparing to the earlier 35/24th passage (Fig.2). As it is clearly seen in Fig.2, the amount of MGMT protein in the cells of G1-T subline at the 66/17th and 66/35th passages did not differ significantly. The mentioned amount exceeded the one after 12.5-dpc embryos of BALB/c mouse strain and was significantly higher than the amount of the investigated protein in the cells of original G1 cell line and its subline G1-OA at the 27th and 35/24th passages respectively.

Chromosomal aberrations were shown to be secondary DNA damages, which occur in two cycles after replication of DNA from O^6MeG at MGMT deficiency [7, 8]. For instance, inhibition of MGMT activity by O^6-benzylguanine in human fibroplasts L136 results in the increase of number of chromosomal aberrations both directly after treatment and in further cell cycles [9]. At the same time CHO cells with overexpressed *Mgmt* acquired resistance to alkylation, which was demonstrated with the formation of exchanges between sister chromatids and chromosomal aberrations, compared to MGMT-deficient cells [10]. Higher frequencies of exchanges between sister chromatids in the second mitosis after MNNG (N-methyl-Nr-nitro-N-nitrosoguanine) treatment, comparing to the first mitosis at exhaustion of MGMT, were revealed by cytogenetic investigation of mouse fibroblasts. It has been determined that the increased number of chromosomal aberrations was induced by primary damages of DNA by O^6MeG [8]. It is predominantly in the works dedicated to the studies on reparative enzyme MGMT in the formation of secondary DNA damages, which include chromosomal aberrations, the main part is given to the chromosomal aberrations, which occur after

treatment with methylating agents, in the cells with different level of *Mgmt* expression. We have analyzed the relative level of *Mgmt* expression in spontaneously immortalized mouse cell line G1 at different passages of *in vitro* cultivation, as well as in the cells of sublines G1-OA and G1-T using Western blot analysis.

The results revealed the highest level of *Mgmt* expression in the cells of subline G1-T, which is specific for multilayer growth [13]. The lowest amount of MGMT protein was revealed in extracts of G1-OA subline cells, which are capable of growing in the poor medium [13]. Preliminary results of cytogenetic analysis of different sublines of G1 cell line, revealed G1-T subline having the lowest number of chromosomal aberrations at the $66/30^{th}$ passage, while G1-OA subline cells revealed the highest frequencies of chromosomal aberrations, micronuclear and multinuclear cells at different passages [11, 13]. In the cells of both original cell line G1and its subline G1-OA, the increase in the amount of DNA repair protein at later passages of *in vitro* cultivation has been revealed. Investigated cell cultures were detected to have the increased genetic instability and destabilized karyotype at early passages. Reduced amount of MGMT protein is in good correlation with the increased number of chromosomal aberrations in the investigated cells. Therefore, the results obtained allow the supposition on the possible role of MGMT deficiency at the increased level of chromosomal aberrations in G1-OA subline.

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А. П. Яцышина, В. В. Лыло, О. В. Пидпала, Т. П. Рубан, И. Н. Вагина, Л. Л. Лукаш

Экспрессия O^6-метилгуанин-ДНК метилтрансферазы в клетках спонтанно иммортализованной линии G1 мыши и ее сублиний G1-OA и G1-T

Резюме

С помощью Вестерн-блот анализа проанализирована экспрессия гена Mgmt в клетках спонтанно иммортализованной линии G1 мыши и ее сублиний G1-OA и G1-T на разных пассажах культивирования in vitro. Самый высокий уровень экспрессии выявлен в клетках сублинии G1-T и наименьший – в клетках сублинии G1-OA. В клетках как исходной клеточной линии мыши G1, так и ее сублинии G1-OA наблюдали увеличение количества репаративного белка MGMT на более поздних пассажах культивирования in vitro. Поскольку для сублинии G1-OA характерны самые высокие частоты хромосомных аберраций, микроядерных и многоядерных клеток, можно предположить возможную роль дефицита репаративного фермента MGMT в повышении уровня хромосомных аберраций в сублинии G1-OA.

Ключевые слова: клетки млекопитающих in vitro, O^6-метилгуанин-ДНК метилтрансфераза (MGMT), хромосомные аберрации.

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