ISSN 0233–7657. Biopolymers and Cell. 2013. Vol. 29. N 2. P. 99–106 doi: 10.7124/bc.00080C

UDC 577.21:577.218

MGMT expression: insights into its regulation. 1. Epigenetic factors

A. P. Iatsyshyna

Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680

a.p.iatsyshyna@imbg.org.ua

O^6-methylguanine-DNA methyltransferase (MGMT) is the DNA repair enzyme responsible for removing of alkylation adducts from the O^6-guanine in DNA. Despite MGMT prevents mutations and cell death, this enzyme can provide resistance of cancer cells to alkylating agents of chemotherapy. The high intra- and inter-individual variations in the human MGMT expression level have been observed indicating to a complicated regulation of this gene. This review is focused on the study of epigenetic factors which could be potentially involved in regulation of the human MGMT gene expression. These include chromatin remodeling via histone modifications and DNA methylation of promoter region and gene body, as well as RNA-based mechanisms, alternative splicing, protein post-translational modifications, and other.

Keywords: O^6-methylguanine-DNA methyltransferase (MGMT), epigenetic regulation of gene expression, methylation, chromatin remodeling.

Introduction. O^6-methylguanine-DNA methyltransferase (MGMT) is the DNA repair enzyme responsible for removing alkylation adducts from the O^6-position of guanine in DNA by mechanism of so-called «suicidal» reaction [1–3]. MGMT is irreversibly inactivated after binding of alkyl group to cysteine, so the synthesis of molecules of MGMT *de novo* is required for further DNA repair. Despite this enzyme prevents mutations and cell death, it can provide resistance of cancer cells to alkylating agents, which are frequently used in the chemotherapy of many types of cancer [3, 4]. Thus, the expression of *MGMT* and its activity in human tumors can determine cell response to therapies with alkylating agents. However, levels of the *MGMT* expression are highly variable among normal tissues within an individual, among different tumors, between cells within a tissue or tumor, as well as between individuals within one tissue [2, 5]. The *MGMT* expression variation with reversible loss has been also revealed during long-term *in vitro* cultivation of cells [5–9].

© Institute of Molecular Biology and Genetics, NAS of Ukraine, 2013

Observed variations of the *MGMT* expression level indicate a complicated regulation of this gene, but molecular basis of intra- and inter-individual variations is still not fully defined. Therefore, the aim of this paper is to review epigenetic factors which could be potentially involved in regulation of the human *MGMT* expression.

This article is the first part of a thematic series on regulation of *MGMT* expression and is devoted to the epigenetic regulation. The next two parts are about genetic factors regulating *MGMT* expression.

Epigenetic regulation of gene expression includes several mechanisms, in particular DNA methylation, histone packaging and modifications, RNA-based mechanisms, post-translational modifications of protein, and other factors. To date, the Encyclopedia of DNA Elements (ENCODE) Consortium aims to build a comprehensive data list of functional regulatory elements in the human genome which is freely available for download and analysis [10]. Most of discussed in this review experimental data, related to regulatory elements within human MGMT, were taken from ENCODE project

at the UCSC Genome Browser (the University of California Santa Cruz, http://genome.ucsc.edu/), Genome Reference Consortium Human Build 37 (GRCh37, or hg19 assembly, submitted in February 2009) [10, 11].

O^6-alkylguanine-DNA alkyltransferases. During lifespan cells are under the influence of different endogenous and exogenous stress factors, which can damage the genomic DNA including the alkylation. The various sites of alkylation have been revealed, but alkylation of guanine at the O^6-position, in despite of its minority, is cytotoxic, has the strongest mutagenic potential, as well as can cause tumor development [2, 12, 13]. Different pathways of DNA repair were evolved in mammalian cells for self-defence from toxic and mutagenic effects [14]. O^6-Alkylguanine-DNA alkyltransferase (official name O^6-methylguanine-DNA methyltransferase, MGMT) is the DNA repair enzyme responsible for removing alkylation adducts from DNA [1–3]. The alkyltransferases are widely expanded among over 100 different species from *Archaea* and *Bacteria* to many organisms of *Eukarya* including humans, but not in *Plantae*, fission yeast *Schizosaccharomyces pombe* and bacterium *Deinococcus radiodurans* [15]. At the same time MGMT is highly conservative that evidences this alkyltransferase has a great importance for genomic integrity (see alignments of sequences of the alkyltransferase and alkyltransferase like proteins in reviews [5, 15]).

This enzyme belongs to the direct repair pathway, it removes alkyl groups (such as methyl-, ethyl-, chloroethyl-group a. o.) without DNA lesion transferring them to an own cysteine residue by mechanism of the so-called «suicidal» reaction, namely MGMT is irreversibly inactivated after binding alkyl group to cysteine, so the synthesis of MGMT molecules *de novo* is required for further DNA repair. The capacity of cells to repair the O^6-alkylguanine depends on the level of MGMT in cell or the rate at which a cell can synthesize this enzyme. Though MGMT prevents mutations and cell death, this enzyme can provide resistance of cancer cells to alkylating agents, which are frequently used in the chemotherapy of many types of cancer [3, 4].

Thus, the expression of MGMT and its activity in human tumors can determine cell response to therapies with alkylating agents.

Intra- and inter-individual variations of *MGMT* expression. The *MGMT* gene is ubiquitously expressed in mammals, but the level of its expression widely varies depending on the type of cell or tissue, cell cycle phase, developmental stage of organism, as well as on species [2, 5, 16]. For example, a high level of the inter- and intra-individual variability of the *MGMT* expression has been revealed in peripheral blood mononuclear cells from healthy individuals [17]. A substantial variation of the amount of MGMT activity has been also revealed in all examined normal and tumor tissues from rats and humans, as well as individual MGMT variations in the human samples [16]. Variation of the MGMT activity level in human normal and tumor tissues, as well as in human lymphocytes is reviewed in [5].

The tissue-specific variation of the human MGMT activity has been shown. For example, the highest level of expression was usually detected in the liver, followed by the colon and lung, and low – in the brain and myeloid tissues [5, 16, 18], while the lowest expression was detected in hematopoietic ($CD34^+$) progenitor cells [2]. In tumors the highest level of *MGMT* expression was observed in the breast, colon, lung and ovarian tumors, and the lowest – in the gliomas, malignant melanomas, pancreatic carcinomas and testis tumors [2].

The *MGMT* expression is widely varied in immortalized mammalian cells. It has been supposed that this variability in cultered cells reflects the *MGMT* expression level in the tissue from which they were obtained [5, 8]. For example, such correlation was revealed for the cell lines of lymphoblastoid cells derived from the peripheral blood lymphocytes [6]. Moreover, it has been shown that levels of the MGMT vary during long-term *in vitro* cultivation of cells [6, 9], and decrease in the *MGMT* expression can be reversible [7]. In particular, the lymphoblastoid cell lines are loosing the MGMT activity compared with the cells at early passages of *in vitro* cultivation [8]. Cell cycle dependent variation of MGMT in cultured mammalian cells is discussed in [5]. In our investigations fluctuations of MGMT protein level during long-term *in vitro* cultivation of the cell lines derived from mouse embryonic germ cells has been also revealed [9].

Epigenetic regulation of the *MGMT* expression. Observed variations of the *MGMT* expression indicate a complicated regulation of this gene. Genetic, epigenetic and environmental factors may affect the gene expression, and the human *MGMT* gene is no exception.

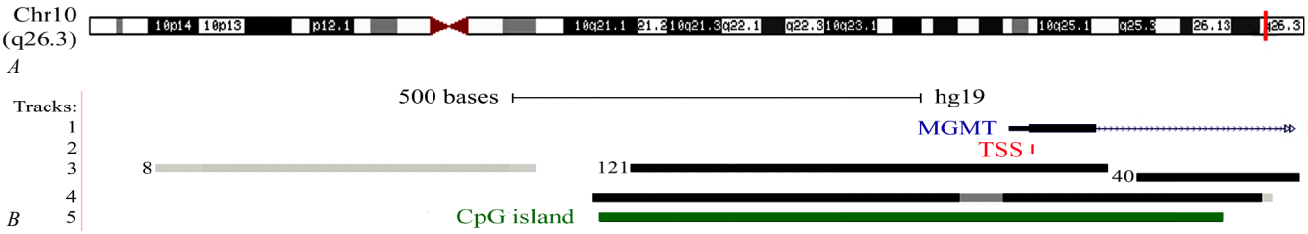

Fig. 1. *Cis*-regulatory elements within the human *MGMT* promoter region: *A* – chromosome 10 ideogram; *B* – integrated regulation tracks from ENCODE (*1* – RefSeq Gene; *2* – the location of TSS of the human *MGMT* gene on track from SwitchGear Genomics (CHR10_P0807_R1); *3* – DNaseI hypersensitive regions, marked as gray and dark boxes, darkness of which is proportional to the maximum signal strength observed in any cell line; the number to the left of the box shows how many cell lines are hypersensitive in the region; *4* – the track of transcription factor ChIP-seq, which shows regions where TFs bind to DNA as assayed in different cell lines; the darkness of the box is proportional to the maximum signal strength observed in any cell line; *5* – CGI, shown as dark green box)

Epigenetic changes can concern chromatin structure via histone modifications and methylation of promoter region and gene body, as well as RNA interference, alternative splicing, and post-translational modifications of the MGMT protein.

DNA methylation and chromatin remodeling. The MGMT promoter contains the CpG island (CGI). A predicted CGI is located at position chr10: 131264949-131265710 in hg19/Human [10]. The size of CGI is 762 bps. It contains 75 CpG dinucleotides and overlaps with the transcription start site (TSS) and 5'-flanking sequence of the gene (Fig. 1). The location of TSSs helps to define the promoter regions. TSS of the human *MGMT* gene (Fig. 1) was determined by Switch Gear Genomics by integrating experimental data using an empirically derived scoring function [10]. This TSS (CHR10_P0807_R1) has position chr10:131265479 in the plus-strand DNA of 10q26 chromosome band, whereas the RefSeq *MGMT* gene is located at chr10: 131265454-131565783 (NM_002412) [10].

CGIs are typically common near or overlapping TSSs and may be associated with promoter regions [19]. In case of the *MGMT* promoter TSS is located within detected CGI. Approximately 70 % of promoters of vertebrates contain CGI, mainly they are promoters of virtually all housekeeping genes, as well as a part of tissue-specific genes and developmental regulator genes [20]. According to promoter types indicated in [19], so-called housekeeping genes with broad expression throughout organismal cycle have promoters of ubiquitous type, which are characterized by dispersed TSSs and ordered nucleosome configuration; they are TATA-depleted and have CGIs. The promoter region of human *MGMT* gene is known to be GC-rich and TATA-free [21]. Since promoters function as sites of transcription initiation by binding and correct positioning of the transcription initiation complex [19], the binding of transcription factors (TFs) with cis-regulatory elements within promoter alters the local chromatin structure, creating open chromatin regions [22]. These regions can be identified by deoxyribonuclease I (DNaseI) hypersensitive site (DHS) mapping [22]. DHS tracks in Fig. 1 display the location of active cis-regulatory elements identified as open chromatin within promoter region of the *MGMT* gene in different cell lines [10]. Three DNase clusters are shown from this track in Fig. 1 [10].

Thus, as shown in Fig. 1, the promoter region of human *MGMT* gene contains TSS, which is located within CGI, DHS and exon 1 of the gene. Overlapping of CGI and DHS marks the open chromatin region and the location of active *cis*-regulatory elements. The ENCODE studies of different cell lines demonstrated that *MGMT* promoter can be targeted by several TFs in this region (Fig. 1). The track of TF ChIP-seq shows regions where TFs bind to DNA as assayed by ChIP-seq (chromatin immunoprecipitation (ChIP) assay in combination with sequencing).

The track in Fig. 2, *B* (see inset), shows nucleosome position by MNase-seq in the *MGMT* promoter region in K562 cell line [10]. DHS, which overlaps with CGI and TFs binding regions, lies in a linker DNA area and is framed by nucleosomes (Fig. 1, Fig. 2, see inset), thus, providing accessibility of DNA to regulatory proteins in the cells expressing gene.

Figures to article by Iatsyshyna A. P.

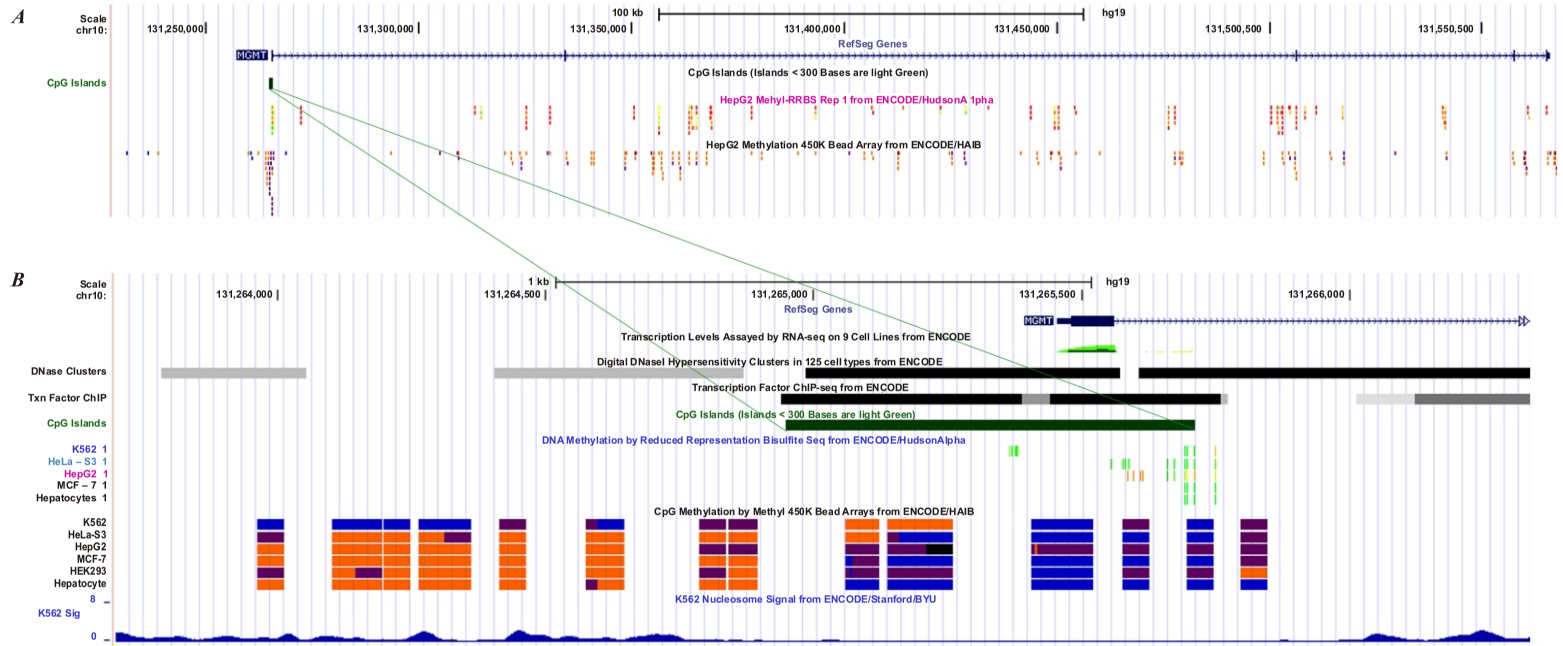

Fig. 2. Methylation data tracks of the human *MGMT* gene from ENCODE project [10]: *A* – methylation data on the gene in HepG2 cells; *B* – methylation status of promoter region. Methylation tracks display the methylation status of specific CpG dinucleotides identified by Reduced Representation Bisulfite Sequencing (RRBS, methylation is represented with an 11–color gradient using the following convention: red – 100 % of molecules sequenced are methylated, yellow – 50 %, green – 0 %) and by the Illumina Infinium Human Methylation 450 Bead Array platform (methylation status is color-coded as: orange – methylated, purple – partially methylated, bright blue – unmethylated) The track of transcription levels is shown for HeLa, HepG2 and K562 cell lines as assayed by high-throughput sequencing of polyadenylated RNA (RNA-seq). Each of the cell lines is associated with a particular color, in particular yellow – HeLa-S3, green – HepG2, blue – K562. DNaseI hypersensitivity clusters are marked as grey and dark boxes, darkness of which is proportional to the maximum signal strength observed in any cell line. The track of TF ChIP-seq shows regions where TFs bind to DNA. CGI is marked as dark box. The track of Nucleosome Signal by MNase-seq is shown for K562 cells

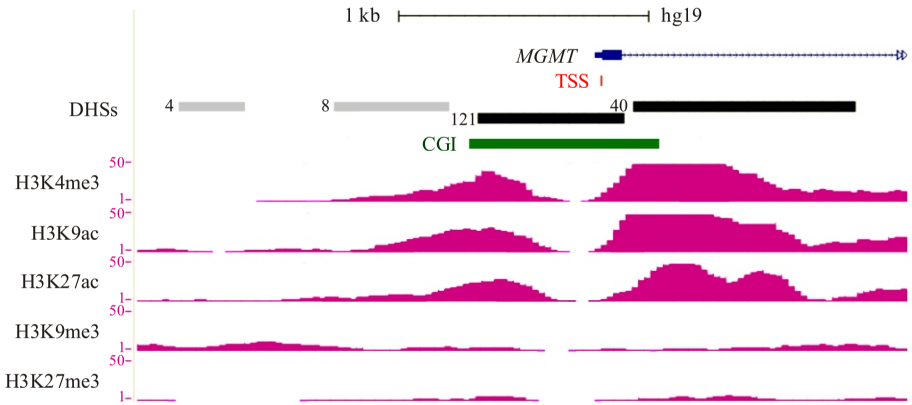

Fig. 3. Histone Modifications within the human MGMT promoter region in HepG2 cells by ChIP-seq from ENCODE/Broad Institute [10]. RefSeq human MGMT gene is marked by dark blue color. The location of TSS on track from SwitchGear Genomics is marked by red. DNaseI hypersensitive sites (DHSs) are marked as gray and dark boxes, darkness of which is proportional to the maximum signal strength observed in any cell line. The number to the left of the box shows how many cell lines are hypersensitive in the region. CGI is shown as dark green box. Histone modification tracks are colored in pink. H3K4me3, H3K9ac, and H3K27ac are associated with transcriptional initiation and open chromatin structure, and mark active or poised to be activated promoters. H3K9me3 and H3K27me3 are associated with repressive heterochromatic state (mark silenced chromatin regions).

ISSN 0233-7657. Biopolymers and Cell. 2013. Vol. 29. N 2

It is well known that DNA methylation at the CGI of promoters plays a key role in the epigenetic silencing of tumor suppressor genes. It was shown that *MGMT* is epigenetically silenced in various human cancer types, too. It is demonstrated on an example of more than 500 primary human tumors, cancer cell lines, and normal tissues that hypermethylation of the *MGMT* promoter, associated with the loss of expression, is frequent [23, 24]. Among examined human neoplasias, the *MGMT* hypermethylation was found in about 40 % of brain tumors and colorectal carcinomas. Approximately 25 % of non-small cell lung carcinomas, lymphomas, and head and neck carcinomas also showed the *MGMT* hypermethylation, while some types of tumors had infrequent the *MGMT* promoter methylation (e. c. pancreatic carcinomas, melanomas, renal carcinomas, acute leukemias, bladder carcinomas) or had not at all the *MGMT* methylation in other cancer types (e. c. carcinomas of breast, endometrium, ovary, liver, small cells of lung) [24, 25].

It has been shown that immortal cell lines with the methylated *MGMT* promoter are more sensitive to alkylating agents [26], and the MGMT-deficient tumors with the methylated promoter also show an increased sensitivity to such drugs [27]. The *MGMT* promoter methylation was revealed to be associated with the tumor regression and prolonged overall and disease-free survival of 40 % of patients with gliomas, which had the methylated promoter and were treated with the alkylating agent carmustine (or BCNU) [27]. The *MGMT* promoter hypermethylation was shown to correlate with a statistically significant increase in survival of patients with diffuse large B-cell lymphoma after treatment with cyclophosphamide as part of multidrug regimen, too [28]. Thereby, it seems that the *MGMT* promoter methylation can be a useful clinical predictive marker of the responsiveness of tumors to alkylating agents and patient survival [28–30]. However, the *MGMT* gene silencing in tumors causes an accumulation of point mutations resulting in genomic instability and determining disease outcome [31]. For example, it has been revealed that the *MGMT* promoter methylation was significantly associated with point mutations of the *K-ras* gene in patients with gastric carcinoma, as well as with patient survival [32].

Methylation of cytosines in the body of the *MGMT* gene and in its promoter results in opposite effects. For example, methylation of promoter region is associated with the loss of MGMT expression [23, 33, 34], whilst methylation of downstream sequences in the gene body correlates with an increased expression [33, 35, 36]. It has been shown that most gene bodies are CpG-poor and extensively methylated, that is a feature of transcribed genes and is not associated with repression of transcription elongation [37]. The *MGMT* body does not contain any predicted CGI (accordingly to the general CGI criteria) and is methylated, for example, in HepG2 cell line expressing this gene (Fig. 2, *A*, see inset), and this observation is consistent with a feature of actively transcribed genes [37]. Possible functions of the gene body methylation are discussed in [37].

Also, it has been demonstrated that the methylation of CGI of *MGMT* is associated with the gene suppression, and it is generally incomplete, localizing in a core promoter region around TSS, creating hot spots of methylation [23].

Chromatin structure related to the methylation of the *MGMT* promoter. Silencing the CGI-containing genes is correlated with increased cytosine methylation, closed chromatin structure, and exclusion of TF binding in the CGI of promoters [37]. Many studies indicate that the methylation of CpGs represses the transcription via recruitment of histone deacetylase complexes by methyl-CpG binding proteins, and as consequence the chromatin condensation [37]. It has been reported that histone deacetylation plays a role in the *MGMT* silencing, too [38, 39].

The importance of histone modifications in epigenetic regulation of the human *MGMT* gene, such as acetylation and methylation of residues, due to chromatin structure changes was shown in many studies. In particular, inactivation of the *MGMT* gene transcription was demonstrated to be associated with the loss of open chromatin structure and exclusion of TFs from Sp1-like binding sites within the CGI, but not with methylation of the TF binding region [40]. The chromatin condensation after CGI methylation in promoter region and consequent complete blockage of the *MGMT* gene expression were shown in other studies by using a luciferase reporter system [38], an analysis of accessibility of restriction enzymes to the *MGMT* promoter [41, 42] a. o. All of them are consistent with a model of methylation-related silencing of gene expression, which involves

binding of methyl-CpG binding protein to methylated DNA, recruitment by this protein of histone deacetylase complexes, and as consequence histone deacetylation and chromatin condensation [38].

It has been shown by using ChIP assay that a higher level of acetylation of histones H3 and H4 bound to the promoter region was associated with the *MGMT* gene expression, while transcriptional inactivation of the gene was caused by formation of condensed chromatin after binding of a greater amount of methyl-CpG binding domain containing proteins (such as MeCP2, MBD1, and CAF-1) to the methylated promoter and histone deacetylation [39].

It has been revealed that associated with open chromatin and active transcription acetylated H3K and H4K, as well as methylated H3K4, were localized outside of the unmethylated CGI whithin minimal promoter in the *MGMT*-expressing cells, whilst closed chromatin was associated with methylated CGI and hypermethylated lysine 9 in histone H3 (H3K9) throughout this island [43, 44]. This observation is consistent with a model for the aberrant silencing of the human *MGMT* gene, in accordance with which the open chromatin structure of CGI in *MGMT*-expressing cells consists of an approximately 250-bp nucleosome-free, TF binding and nuclease-accessible region of DNA, and is formed by at least four flanking precisely positioned nucleosome-like structures [42]. Such positioning of nucleosomes is lost and random in *MGMT* non-expressing cells causing the closed chromatin structure [42]. Thus, in *MGMT* non-expressing cells the closed chromatin structure is associated with methylated CGI and hypermethylated H3K9 throughout this island [43]. Dimethylation of H3K9 and binding of methyl-CpG binding protein have been shown to be common and essential for *MGMT* silencing in cases with hypomethylated promoter region [44].

An example of open chromatin state is shown in Fig. 3 (see inset). Histone modification tracks from ENCODE project [10] display signals for markers of active promoter, such as H3K4me3, H3K9ac, and H3K27ac in *MGMT*-expressing HepG2 cells, whereas there is no signal for markers of silenced chromatin regions (H3K9me3 and H3K27me3).

A causality between the *MGMT* promoter methylation and its transcriptional silencing was demonstrated also in cultured cells [26, 45, 46]. It was shown that logarithmically growing normal human fibroblasts displayed approximately 15 % of CpG dinucleotide methylation in CGI of the *MGMT* promoter, compared with approximately 50 % of CGI methylation in confluent growth-arrested cells [45]. The *MGMT* promoter methylation was shown to be not permanent and reversed at logarithmic growth of cells [45]. Progressive increasing of the CGI methylation of the *MGMT* promoter region was demonstrate with increasing of cell culture passage number, so established immortalized cell lines often completely lack of the gene expression [26, 46]. These findings probably could explain our and other researchers observations of fluctuations of MGMT levels at different stages of *in vitro* cultivation and establishment of cell lines [9].

Conserved sites for miRNA predicted in the 3'UTR of the human MGMT transcript

miRNA	Position in 3'UTR	Seed match
hsa-miR-1197	96–102	7mer-m8
hsa-miR-4436a	97–103	7mer-m8
hsa-miR-3607-3p	138–144	7mer-m8
hsa-miR-4718	140–146	7mer-1A
hsa-miR-4539	152–159	8mer
hsa-miR-3121-3p	209–215	7mer-m8
hsa-miR-3911	362–368	7mer-m8

RNA interference is evolutionarily conserved and widespread mechanism regulation of gene expression by small noncoding RNAs [47, 48]. MicroRNAs (miRNAs) have been identified as one of the most widespread class of endogenous small RNAs in mammalian cells [48]. Sites for many miRNA from different families are predicted in the 3'UTR of MGMT transcript NM_002412 by using TargetScanHuman 5.1 on its webpage [47], as well as in the UCSC Genome Browser (Table). To date there is no evidence about regulation of the human *MGMT* gene expression *in vivo* via RNA interference mechanism, but silencing of the MGMT protein biosynthesis via RNA interference belongs to the strategies to increase the sensitivity of cancer cells to alkylating drugs. Small double-stranded RNA molecules can be introduced exogenously as short interfering RNAs [14]. The clinical use of such strategy is suggested, but it is prevented by significant problems,

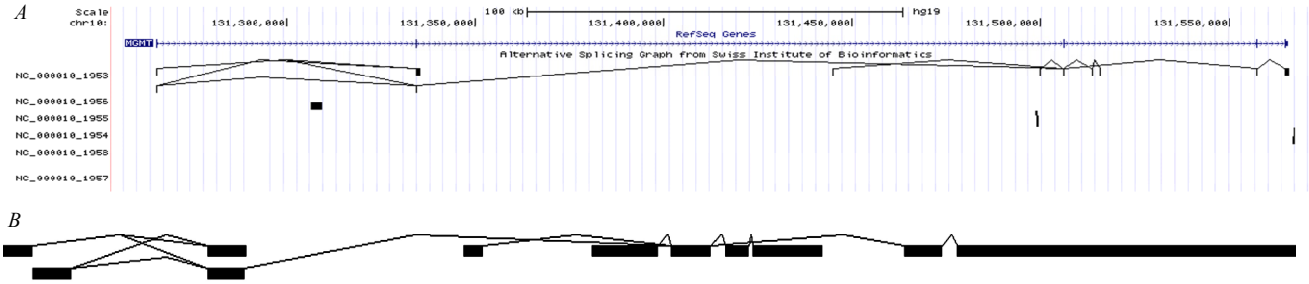

Fig. 4. Graphs of predicted alternative splicing transcripts from Swiss Institute of Bioinformatics [10]. (NC_000010_1953, chr10:131265453-131566301): *A* – plot of the alt-splicing drawn to scale; *B* – plot of alt-splicing drawn with exons enlarged. The graphs of predicted alternative splicing transcripts are constructed by analyzing experimental RNA transcripts. The black blocks represent exons; lines indicate introns

among which are incomplete silencing of a target gene and so-called off-target effects, non-specific immune responses, and a major challenge – *in vivo* delivery.

A l t e r n a t i v e s p l i c i n g of genes is an important mechanism of the post-transcriptional regulation of gene expression in metazoan, generating different transcripts from a single pre-mRNA [49]. The alternative *MGMT* transcripts were predicted by Swiss Institute of Bioinformatics (NC_000010_1953). The UCSC alternative splicing track is constructed by analyzing experimental RNA transcripts (Fig. 4), but no one alternatively spliced isoform was transcribed from the *MGMT* gene [50] according to the Alternative Splicing Annotation Project [51]. Thus, the expression of alternative *MGMT* transcripts must be shown in experiments of transcriptome profiling by using not only hybridization-based microarrays, but next generation sequencing based approach, RNA-seq, and so on [52].

Post-translational modifications of the MGMT protein and their influence upon the enzyme fate are extensively reviewed and discussed in [50].

Conclusions. The DNA repair enzyme MGMT belongs to the direct reversal repair system, i. e. removes alkyl groups from the O^6-position guanine without DNA lesion by mechanism of so-called «suicidal» reaction, and the capacity of cells to repair O^6-alkylguanine depends on the level of MGMT in cell or the rate at which a cell can synthesize it. However, MGMT not only prevents mutations and cell death, but the enzyme can provide resistance of cancer cells to alkylating chemotherapy. The high intra- and inter-individual variations in the human *MGMT* expression level have been observed indicating a complicated regulation of this gene. In this study the epigenetic factors, which could be potentially involved in regulation of the *MGMT* expression, have been reviewed. Among them are DNA methylation of promoter region and gene body, chromatin remodeling via histone modifications, RNA interference, alternative splicing, and protein post-translational modifications. It has been shown that methylation of the *MGMT* promoter is often associated with the loss of its expression in tumor cells, whilst methylation of the gene body correlates with an increased expression. The data on methylation of the *MGMT* promoter and gene body in many different cell lines from ENCODE are consistent with the obtained data, providing additional indirect evidence of a control function of these epigenetic factors in the *MGMT* transcription. Histone modification markers of open chromatin structure have been also revealed within promoter region of different *MGMT*-expressing cells. This promoter region spans the CGI and DHS, contains TSS and binding sites for several TFs. Also, histone markers of closed chromatin have been revealed within methylated promoter in cells not expressing this alkyltransferase. Whereas predicted within 3'UTR of *MGMT* sites for many miRNA, as well alternative transcripts have no experimental evidence and are needed to be analyzed.

Acknowledgement. The study was supported by grant for Young Scientists from the National Academy of Sciences of Ukraine (0111U008220).

А. П. Яцишина

Експресія гена *MGMT*: розуміння її регуляції.
1. Епігенетичні фактори

Резюме

O^6-метилгуанін-ДНК метилтрансфераза (MGMT) – це репаративний фермент, який видаляє алкільні адукти з O^6-гуаніну в ДНК. Незважаючи на те, що MGMT запобігає появі мутацій і клітин-

ній смерті, він також забезпечує стійкість ракових клітин до алкілувальних сполук за хіміотерапії. Спостерігають значні внутрішньо- та міжіндивідуальну коливання у рівнях експресії MGMT, що вказує на складну систему регуляції даного гена. Представлений огляд присвячений вивченню епігенетичних факторів, які можуть бути потенційно залучені до регуляції експресії гена MGMT людини. До них належать ремоделювання хроматину за рахунок модифікацій гістонів і метилювання ДНК промоторної ділянки та тіла гена, а також РНК-регуляторні механізми, альтернативний сплайсинг, посттрансляційні модифікації білка тощо.

Ключові слова: О⁶-метилгуанін-ДНК-метилтрансфераза (MGMT), епігенетична регуляція експресії гена, метилювання, ремоделювання хроматину.

А. П. Яцышина

Экспрессия гена *MGMT*: понимание ее регуляции.

1. Эпигенетические факторы

Резюме

O^6-метилгуанин-ДНК метилтрансфераза (MGMT) – это фермент репарации ДНК, ответственный за удаление алкильных аддуктов из O^6-гуанина в ДНК. Несмотря на то, что MGMT предохраняет от появления мутаций и клеточной гибели, этот фермент может также обеспечивать устойчивость раковых клеток к алкилирующим соединениям при химиотерапии. Обнаружены высокие внутри- и межиндивидуальные вариации в уровнях экспрессии MGMT, что указывает на сложную систему регуляции этого гена. Данный обзор посвящен изучению эпигенетических факторов, которые потенциально могут участвовать в регуляции экспрессии гена MGMT человека. Среди них ремоделирование хроматина с помощью модификации гистонов и метилирования ДНК промоторного участка и тела гена, а также РНК-регуляторные механизмы, альтернативный сплайсинг, посттрансляционные модификаии белка и др.

Ключевые слова: O^6-метилгуанин-ДНК метилтрансфераза (MGMT), эпигенетическая регуляция експрессии гена, метилирование, ремоделирование хроматина.

REFERENCES

1. *Pegg A. E., Dolan M. E., Moschel R. C.* Structure, function, and inhibition of O^6-alkylguanine-DNA alkyltransferase // Prog. Nucleic Acid Res. Mol. Biol.–1995.–**51**.–P. 167–223.
2. *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.
3. *Verbeek B., Southgate T. D., Gilham D. E., Margison G. P.* O^6-Methylguanine-DNA methyltransferase inactivation and chemotherapy // Br. Med. Bull.–2008.–**85**.–P. 17–33.
4. *Hegi M. E., Liu L., Herman J. G., Stupp R., Wick W., Weller M., Mehta M. P., Gilbert M. R.* Correlation of O^6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate *MGMT* activity // J. Clin. Oncol.–2008.–**26**, N 25.–P. 4189–4199.
5. *Margison G. P., Povey A. C., Kaina B., Santibanez-Koref M. F.* Variability and regulation of O^6-alkylguanine-DNA alkyltransferase // Carcinogenesis.–2003.–**24**, N 4.–P. 625–635.
6. *Sagher D., Karrison T., Schwartz J. L., Larson R. A., Strauss B.* Heterogeneity of O^6-alkylguanine-DNA alkyltransferase activity in peripheral blood lymphocytes: relationship between this activity in lymphocytes and in lymphoblastoid lines from normal controls and from patients with Hodgkin's disease or non-Hodgkin's lymphoma // Cancer Res.–1989.–**49**, N 19.–P. 5339–5344.
7. *Arita I., Fujimori A., Takebe H., Tatsumi K.* Evidence for spontaneous conversion of Mex^- to Mex^+ in human lymphoblastoid cells // Carcinogenesis.–1990.–**11**, N 10.–P. 1733–1738.
8. *Strauss B. S.* The control of O^6-methylguanine-DNA methyltransferase (MGMT) activity in mammalian cells: a pre-molecular view // Mutat. Res.–1990.–**233**, N 1–2.–P. 139–150.
9. *Iatsyshyna A. P., Lylo V. V., Pidpala O. V., Ruban T. P., Vagina I. M., Lukash L .L.* The expression of O^6-methylguanine-DNA methyltransferase in the spontaneously immortalized mouse cell line G1 and its sublines G1-OA and G1-T // Biopolym. Cell.–2007.–**23**, N 3.–P. 250–254.
10. *Meyer L. R., Zweig A. S., Hinrichs A. S., Karolchik D., Kuhn R. M., Wong M., Sloan C. A., Rosenbloom K. R., Roe G., Rhead B., Raney B. J., Pohl A., Malladi V. S., Li C. H., Lee B. T., Learned K., Kirkup V., Hsu F., Heitner S., Harte R. A., Haeussler M., Guruvadoo L., Goldman M., Giardine B. M., Fujita P. A., Dreszer T. R., Diekhans M., Cline M. S., Clawson H., Barber G. P., Haussler D., Kent W. J.* The UCSC Genome Browser database: extensions and updates 2013 // Nucleic Acids Res.–2013.–**41**, Database issue.–D64–69.
11. *Kent W. J., Sugnet C. W., Furey T. S., Roskin K. M., Pringle T. H., Zahler A. M., Haussler D.* The human genome browser at UCSC // Genome Res.–2002.–**12**, N 6.–P. 996–1006.
12. *Pegg A. E.* Methylation of the O^6 position of guanine in DNA is the most likely initiating event in carcinogenesis by methylating agents // Cancer Invest.–1984.–**2**, N 3.–P. 223–231.
13. *Singer B.* Alkylation of the O^6 of guanine is only one of many chemical events that may initiate carcinogenesis // Cancer Invest.–1984.–**2**, N 3.–P. 233–238.
14. *Iatsyshyna A.* Current approaches to improve the anticancer chemotherapy with alkylating agents: state of the problem in world and Ukraine // Biopolym. Cell.–2012.–**28**, N 2.–P. 83–92.
15. *Tubbs J. L., Pegg A. E., Tainer J. A.* DNA binding, nucleotide flipping, and the helix-turn-helix motif in base repair by O^6-alkylguanine-DNA alkyltransferase and its implications for cancer chemotherapy // DNA Repair (Amst).–2007.–**6**, N 8.–P. 1100–1115.
16. *Pegg A. E., Dolan M. E., Scicchitano D., Morimoto K.* Studies of the repair of O^6-alkylguanine and O^4-alkylthymine in DNA by alkyltransferases from mammalian cells and bacteria // Environ. Health Perspect.–1985.–**62**.–P. 109–114.
17. *Janssen K., Eichhorn-Grombacher U., Schlink K., Nitzsche S., Oesch F., Kaina B.* Long-time expression of DNA repair enzymes MGMT and APE in human peripheral blood mononuclear cells // Arch. Toxicol.–2001.–**75**, N 5.–P. 306–312.
18. *Gerson S. L., Trey J. E., Miller K., Berger N. A.* Comparison of O^6-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissues // Carcinogenesis.–1986.–**7**, N 5.–P. 745–749.
19. *Lenhard B., Sandelin A., Carninci P.* Metazoan promoters: emerging characteristics and insights into transcriptional regulation // Nat. Rev. Genet.–2012.–**13**, N 4.–P. 233–245.
20. *Deaton A. M., Bird A.* CpG islands and the regulation of transcription // Genes Dev.–2011.–**25**, N 10.–P. 1010–1022.
21. *Harris L. C., Potter P. M., Tano K., Shiota S., Mitra S., Brent T. P.* Characterization of the promoter region of the human O^6-methylguanine-DNA methyltransferase gene // Nucleic Acids Res.–1991.–**19**, N 22.–P. 6163–6167.
22. *Maurano M. T., Humbert R., Rynes E., Thurman R. E., Haugen E., Wang H., Reynolds A. P., Sandstrom R., Qu H., Brody J., Shafer A., Neri F., Lee K., Kutyavin T., Stehling-Sun S., Johnson A. K., Canfield T. K., Giste E., Diegel M., Bates D., Hansen R. S.,*

Neph S., Sabo P. J., Heimfeld S., Raubitschek A., Ziegler S., Cotsapas C., Sotoodehnia N., Glass I., Sunyaev S. R., Kaul R., Stamatoyannopoulos J. A. Systematic localization of common disease-associated variation in regulatory DNA // Science.–2012.–**337**, N 6099.–P. 1190–1195.

23. *Qian X., von Wronski M. A., Brent T. P.* Localization of methylation sites in the human O^6-methylguanine-DNA methyltransferase promoter: correlation with gene suppression // Carcinogenesis.–1995.–**16**, N 6.–P. 1385–1390.
24. *Esteller M., Hamilton S. R., Burger P. C., Baylin S. B., Herman J. G.* Inactivation of the DNA repair gene O^6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia // Cancer Res.–1999.–**59**, N 4.–P. 793–797.
25. *Herath N. I., Walsh M. D., Kew M., Smith J. L., Jass J. R., Young J., Leggett B. A., Macdonald G. A.* Silencing of O^6-methylguanine DNA methyltransferase in the absence of promoter hypermethylation in hepatocellular carcinomas from Australia and South Africa // Oncol. Rep.–2007.–**17**, N 4.–P. 817–822.
26. *Danam R. P., Howell S. R., Remack J. S., Brent T. P.* Heterogeneous methylation of the O(6)-methylguanine-DNA methyltransferase promoter in immortalized IMR90 cell lines // Int. J. Oncol.–2001.–**18**, N 6.–P. 1187–1193.
27. *Esteller M., Garcia-Foncillas J., Andion E., Goodman S. N., Hidalgo O. F., Vanaclocha V., Baylin S. B., Herman J. G.* Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents // N. Engl. J. Med.–2000.–**343**, N 19.–P. 1350–1354.
28. *Esteller M., Gaidano G., Goodman S. N., Zagonel V., Capello D., Botto B., Rossi D., Gloghini A., Vitolo U., Carbone A., Baylin S. B., Herman J. G.* Hypermethylation of the DNA repair gene O(6)-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma // J. Natl Cancer Inst.–2002.–**94**, N 1.–P. 26–32.
29. *Soejima H., Zhao W., Mukai T.* Epigenetic silencing of the *MGMT* gene in cancer // Biochem. Cell Biol.–2005.–**83**, N 4.–P. 429–437.
30. *Maier S., Dahlstroem C., Haefliger C., Plum A., Piepenbrock C.* Identifying DNA methylation biomarkers of cancer drug response // Am. J. Pharmacogenomics.–2005.–**5**, N 4.–P. 223–232.
31. *Esteller M., Herman J. G.* Generating mutations but providing chemosensitivity: the role of O^6-methylguanine DNA methyltransferase in human cancer // Oncogene.–2004.–**23**, N 1.–P. 1–8.
32. *Park T. J., Han S. U., Cho Y. K., Paik W. K., Kim Y. B., Lim I. K.* Methylation of O(6)-methylguanine-DNA methyltransferase gene is associated significantly with *K-ras* mutation, lymph node invasion, tumor staging, and disease free survival in patients with gastric carcinoma // Cancer.–2001.–**92**, N 11.–P. 2760–2768.
33. *Wang Y., Kato T., Ayaki H., Ishizaki K., Tano K., Mitra S., Ikenaga M.* Correlation between DNA methylation and expression of O^6-methylguanine-DNA methyltransferase gene in cultured human tumor cells // Mutat. Res.–1992.–**273**, N 2.–P. 221–230.
34. *Harris L. C., Remack J. S., Houghton P. J., Brent T. P.* Wild-type p53 suppresses transcription of the human O^6-methylguanine-DNA methyltransferase gene // Cancer Res.–1996.–**56**, N 9.–P. 2029–2032.
35. *Pieper R. O., Costello J. F., Kroes R. A., Futscher B. W., Marathi U., Erickson L. C.* Direct correlation between methylation status and expression of the human O-6-methylguanine DNA methyltransferase gene // Cancer Commun.–1991.–**3**, N 8.–P. 241–253.
36. *Christmann M., Pick M., Lage H., Schadendorf D., Kaina B.* Acquired resistance of melanoma cells to the antineoplastic agent fotemustine is caused by reactivation of the DNA repair gene *MGMT* // Int. J. Cancer.–2001.–**92**, N 1.–P. 123–129.
37. *Jones P. A.* Functions of DNA methylation: islands, start sites, gene bodies and beyond // Nat. Rev. Genet.–2012.–**13**, N 7.–P. 484–492.
38. *Bhakat K. K., Mitra S.* CpG methylation-dependent repression of the human O^6-methylguanine-DNA methyltransferase gene linked to chromatin structure alteration // Carcinogenesis.–2003.–**24**, N 8.–P. 1337–1345.
39. *Danam R. P., Howell S. R., Brent T. P., Harris L. C.* Epigenetic regulation of O^6-methylguanine-DNA methyltransferase gene expression by histone acetylation and methyl-CpG binding proteins // Mol. Cancer Ther.–2005.–**4**, N 1.–P. 61–69.
40. *Pieper R. O., Patel S., Ting S. A., Futscher B. W., Costello J. F.* Methylation of CpG island transcription factor binding sites is unnecessary for aberrant silencing of the human *MGMT* gene // J. Biol. Chem.–1996.–**271**, N 23.–P. 13916–13924.
41. *Costello J. F., Futscher B. W., Kroes R. A., Pieper R. O.* Methylation-related chromatin structure is associated with exclusion of transcription factors from and suppressed expression of the O-6-methylguanine DNA methyltransferase gene in human glioma cell lines // Mol. Cell Biol.–1994.–**14**, N 10.–P. 6515–6521.
42. *Patel S. A., Graunke D. M., Pieper R. O.* Aberrant silencing of the CpG island-containing human O^6-methylguanine DNA methyltransferase gene is associated with the loss of nucleosome-like positioning // Mol. Cell Biol.–1997.–**17**, N 10.–P. 5813–5822.
43. *Nakagawachi T., Soejima H., Urano T., Zhao W., Higashimoto K., Satoh Y., Matsukura S., Kudo S., Kitajima Y., Harada H., Furukawa K., Matsuzaki H., Emi M., Nakabeppu Y., Miyazaki K., Sekiguchi M., Mukai T.* Silencing effect of CpG island hypermethylation and histone modifications on O^6-methylguanine-DNA methyltransferase (*MGMT*) gene expression in human cancer // Oncogene.–2003.–**22**, N 55.–P. 8835–8844.
44. *Zhao W., Soejima H., Higashimoto K., Nakagawachi T., Urano T., Kudo S., Matsukura S., Matsuo S., Joh K., Mukai T.* The essential role of histone H3 Lys9 di-methylation and MeCP2 binding in *MGMT* silencing with poor DNA methylation of the promoter CpG island // J. Biochem.–2005.–**137**, N 3.–P. 431–440.
45. *Pieper R. O., Lester K. A., Fanton C. P.* Confluence-induced alterations in CpG island methylation in cultured normal human fibroblasts // Nucleic Acids Res.–1999.–**27**, N 15.–P. 3229–3235.
46. *Yamada H., Vijayachandra K., Penner C., Glick A.* Increased sensitivity of transforming growth factor (TGF) beta 1 null cells to alkylating agents reveals a novel link between TGFbeta signaling and O(6)-methylguanine methyltransferase promoter hypermethylation // J. Biol. Chem.–2001.–**276**, N 22.–P. 19052–19058.
47. *Friedman R. C., Farh K. K., Burge C. B., Bartel D. P.* Most mammalian mRNAs are conserved targets of microRNAs // Genome Res.–2009.–**19**, N 1.–P. 92–105.
48. *Li Z., Rana T. M.* Molecular mechanisms of RNA-triggered gene silencing machineries // Acc. Chem. Res.–2012.–**45**, N 7.–P. 1122–1131.
49. *Singh R. K., Cooper T. A.* Pre-mRNA splicing in disease and therapeutics // Trends Mol. Med.–2012.–**18**, N 8.–P. 472–482.
50. *Iatsyshyna A. P., Nidoieva Z. M., Pidpala O. V., Lukash L. L.* Bioinformatic analysis of potential post-translational modification sites of the human O^6-methylg uanine-DNA methylt ransferase (MGMT) protein // Ukr. Biokhim. Zh.–2012.–**84**, N 6.–P. 74–85.
51. *Lee C., Atanelov L., Modrek B., Xing Y.* ASAP: the Alternative Splicing Annotation Project // Nucleic Acids Res.–2003.–**31**, N 1.–P. 101–105.
52. *Sanchez-Pla A., Reverter F., Ruiz de Villa M. C., Comabella M.* Transcriptomics: mRNA and alternative splicing // J. Neuroimmunol.–2012.–**248**, N 1–2.–P. 23–31.

Received 30.12.12