UDC 575.224
MACEWICZ L.L., LYLO V.V., KARPOVA I.S., KOTSARENKO K.V., RUBAN T.A., LUKASH L.L.
Institute of Molecular Biology and Genetics of Natl. Acad. Sci. of Ukraine,
Ukraine, 03680, Kyiv, Akad. Zabolotnogo str., 150, e-mail: lukash@imbg.org.ua

PLANT AND ANIMAL LECTINES AS MODULATORS OF *MGMT* AND *MARP* GENE EXPRESSION *IN VITRO*

Repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) is the major defence factor against the mutagenic, carcinogenic and cytotoxic effects of alkylating agents [1, 2]. It can transfer an alkyl group from the O6-position of guanine to its own cysteine residue with further degradation of MGMT molecule [3, 4]. Thus, MGMT protects normal cells from endogenous and exogenous mutagenes and carcinogens. But alkylating agents are widely used in cancer chemotherapy schemes. The MGMT expression and activity in malignant cells are often high, and need to be inhibited. Besides the secondary tumorigenesis after the treatment with alkylating agents is possible. Thus, the protection of non-tumor cells from genotoxic anti-cancer drugs is an issue. Therefore, this enzyme is considered to be one of the targets to regulate antitumor efficacy of alkylating agents [1, 5, 6].

The expression level of MGMT gene can differ widely in individuals, in tissues and organs of the same organism, and in normal and tumor cells of the same organ [7, 8]. In our previous investigations we found and described the unknown protein with M.w. ≈ 50 kDa [9, 10] named as MARP (anti-Methyltransferase Antibody Recognizable Protein), shown to be highly inducible, and presumed to be the part of DNA repair system in mammalian cells. It can be regulated by some cytokines and growth factors [11].

Therefore the search for effective modulators of MGMT activity and expression is actual for improving anti-tumor efficacy of alkylating agents and preventing of their side effects in normal cells. To date only a small number of DNA repair genes have been shown to be inducible in mammalian cells and *MGMT* is presumably one of the best studied ones. And the inducibility of MARP have not been studied yet. According to the literature data the *MGMT* gene expression may be affected by various factors: the alkylating agents, single-strand DNA breaks, transcription factors, activators of protein kinase C, *MGMT* gene promoter hypermethylation, etc. [12]. Regulation of expression can also be indirect through different intracellular signaling pathways [13]. Some cytokines are known to be able to influence on the *MGMT* and *MARP* gene expression as well [1, 13–15].

Plant and animal lectines are recognized as regulating proteins due to their broad spectrum of biological activity in all living systems. Biological functions of these proteins are based on their carbohydrate specificity. Exogenous lectins can influence through glycosylated surface of the cell membrane, and can up- and downregulate adhesion, migration, apoptosis, proliferation etc in pro- and eukaryotic systems [16]. Previously for the first time we have studied the ability of such a lectins to influence the processes of mutagenesis and antimutagenesis in prokaryotic test systems [19] and mammalian cell cultures [18–20]. The elderberry bark lectins were shown to have both mutagenic and toxic activity (at high concentrations) and protective properties (at low concentrations) [18, 21]. In our studies it has also been shown that plant lectins are able to modify DNA damage repair process [22].

Thus, we have assumed that repair enzyme MGMT could also be a target of lectin regulatory impact on mutation process. The aim of our study was to examine the effect of panel of lectins on the MGMT and MARP expression levels in tumor and non-tumor mammalian cells *in vitro*.

Materials and methods

The following cell cultures were used in the experiments: 4BL – fibroblast-like cell line, received in our laboratory from blood of an adult donor; the standard line of Hep2 cells. The cells were cultivated in a standard DMEM growth medium with an addition of 10 % Fetal bovine serum (PAA) and antibiotics (penicillin and streptomycin) at 37°C with 4 % CO_2. As biological factors commercial preparations of elderberry (Sambucus nigra) bark lectins, perch eggs lectins, lentil seed lectins (Lectinotest, Lviv, Ukraine) were used and as a chemical mutagen – nickel chloride. Conditions of cell treatment with lectins and $NiCl_2$ were described previously [22]. Protein extracts were obtained according to a previously described method [15].

SDS electrophoresis of the proteins was performed in 12 % polyacrylamide gel by the Laemmli method [23]. The total protein

concentration was determined by the Bradford method [24] in every sample in order to an equal quantity of the protein was loaded into every lane. Monoclonal antibodies against human MGMT (clone 23.2, isotype IgG2b) were obtained from Novus Biologicals, USA. Secondary antibodies peroxidase conjugated with horseradish were from Sigma. The procedure of MGMT identification in the samples was performed according to the recommendation of the manufacturer of monoclonal antibodies [http://www.novusbio.com/]. For even loading control the densitometry of the stained hybridized membrane was used (Scion Image program) as there are some literature data that total protein stains is an acceptable alternative to single-protein loading controls [25].

Results and discussion

4BL cell culture was treated by panel of lectins including perch egg lectin (PEL), lentil seed lectin (LSL) and elderberry bark lectin (SNA-I) in concentrations 20 and 80 mkg/ml during 4h. With Western-blot analysis it was shown absence of MGMT expression both in control and in treated cells (fig. 1, a), but MARP protein was detected in all the samples. Under the SNA-I treatment MARP expression was increased in cells treated by 20 mkg/ml, while 80 mkg/ml concentration had no effect. The similar tendency was shown in the case of animal lectin (PEL) treated cells but MARP expression level was higher. The effect of LSL treatment has another dose dependence: 20 mkl/ml concentration have no affect MARP expression, but 80 mkg/ml extremely increased the amount of protein. Thus, all lectins studied were shown to influence the MARP expression on protein level, depending on the origin of lectin.

Further we have performed the more detailed study of the dose dependence of PEL treatment effect in 4BL cells (fig. 1, b). PEL was shown to induce MARP expression with all treatment concentrations (0.2, 2 and 20 mkg/ml), but level of expression was the same in all treated samples . Thus, PEL active dose is rather low.

The ability of of SNA-I to induce MARP expression were the lowest in studied lectin panel, and the in some cases were undetectable (fig. 1, c). But, newertheless, such small induction was observed even in samples treated by low 0.2 mkg/ml lectin concentration.

Though the low influence of SNA-I-induced MARP expression level changes in non-tumor intact cells, in our earlier studies we exhibited the DNA repair induction by SNA-I in nickel-damaged cells [21]. And this was the reason to study the joint effect of this lectin and $NiCl_2$ (fig. 2).

So, the moderate up-regulation of MARP expression level in cells treated by 0.2 mkg/ml of lectin, and downregulation of this level in nickel-treated cells was shown. But in cells treated both by nickel chloride and lectin the MARP expression level was the same as in untreated cells. Thus, we can conclude that in normal cell cultures SNA-I lectin modulates the MARP expression rather slightly but we suppose that it can cooperate with some components of repair system after mutagen action.

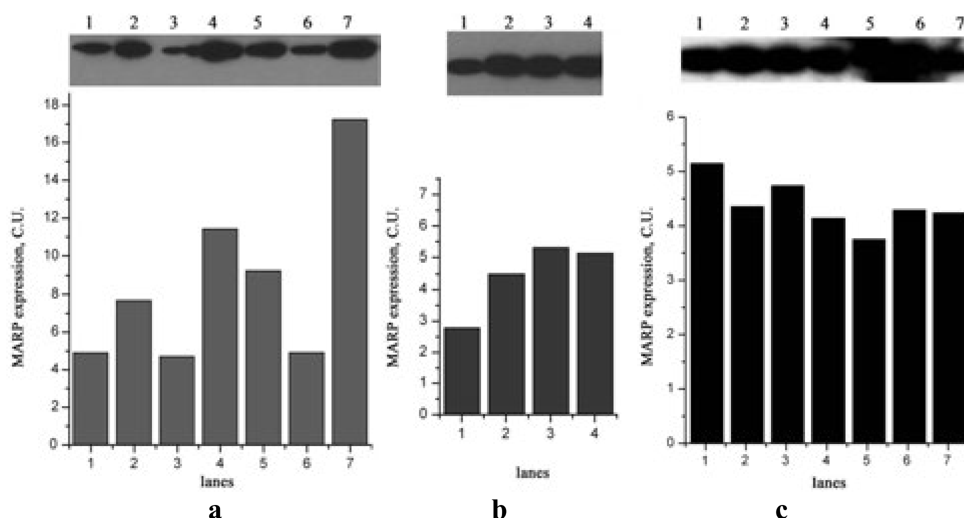

Fig. 1. The MARP expression in 4BL cells treated by perch eggs lectin (PEL), lentil seed lectin (LSL) and elderberry bark lectin (SNA-I). **a)** 1 – control, 2 – SNA-I, 20 mkg/ml, 3 – SNA-I, 80 mkg/ml, 4 – PEL, 20 mkg/ml, 5 – PEL, 80 mkg/ml, 6 – LSL, 20 mkg/ml, 7 – LSL, 80 mkg/ml. **b)** 1 – PEL, control, 2 – PEL, 20 mkg/ml, 3 – PEL, 2 mkg/ml, 4 – PEL, 0.2 mkg/ml. **c)** 1 – SNA-I, control, 2 – SNA-I, 20 mkg/ml, 3 – SNA-I, 2 mkg/ml, 4 – SNA-I, 0.2 mkg/ml, 5 – SNA-I, 20 mkg/ml 72h postincubation, 6 – SNA-I, 2 mkg/ml 72h postincubation, 7 – SNA-I, 0.2 mkg/ml 72h postincubation

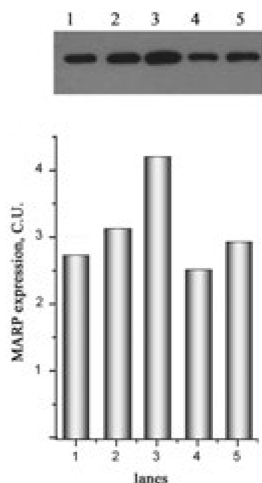

Fig. 2. The MARP expression in 4BL cells treated by elderberry bark lectin (SNA-I) and NiCl2 (500 mkg/ml): 1 – control; 2 – SNA-I, 2 mkg/ml, 3 – SNA-I, 0,2 mkg/ml; 4 – $NiCl_2$; 5 – $NiCl_2$ + SNA-I, 0,2 mkg/ml

Moreover we were interested if SNA-I can change its action in other stress conditions. And in our futher experiments we did demonstrate that in unstable cell culture the SNA-I effect is much more manifested. Cell culture destabilization was induced by cultural medium with high ionic strength, and was accompanied by lost of genomic stability [26]. Enhancing MARP expression in these conditions under the treatment with SNA-I was substantial and persisted at least up to 5 days (fig. 3, a).

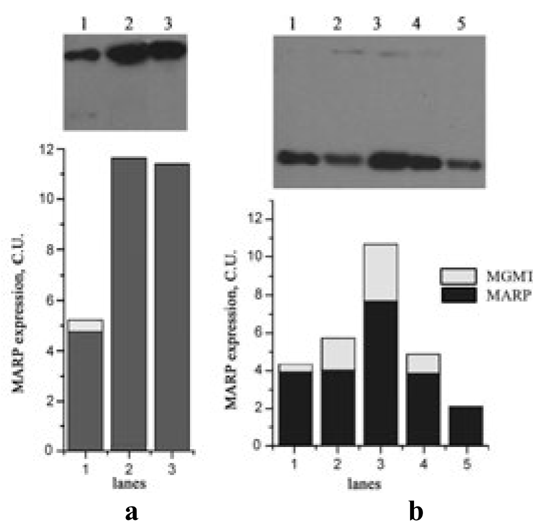

Fig. 3. The MARP expression in destabilized 4BL cells **(a)** and Hep-2 tumor cells **(b)** treated by elderberry bark lectin (SNA-I): a) 1 – control, 2 – SNA-I (20 mkg/ml), 3 – 5 days postincubation after SNA-I (20 mkg/ml) treatment; b) 1 – control, 2 – SNA-I (80 mkg/ml), 3 – SNA-I (20 mkg/ml), 4 – SNA-I (2 mkg/ml), 5 – SNA-I (0.2 mkg/ml)

Substantial influence of SNA-I both on MARP and MGMT expression was demonstrated in cells of tumor origih (Hep-2) (fig. 3, b). But the character of such influence was completely different from the one found in 4Bl. The lowest concentration 0,2 mkg/ml which was shown to be genoprotective in non-malignant cell lines and primary human cells, causes near two-fold decreasing MGMT expression. MARP expression was not detected at all, whilst this enzyme is present in intact Hep-2 cells. In cell culture treated with 2 mkg/ml of lectin the expression levels of both MGMT and MARP are close to the control level, and in 20 mkg/ml treated cells are substantially enhanced. But further increasing lectin concentration leads to decrease of MGMT and MARP expression levels.

As result of this work SNA-I lectin was shown to discriminate non-tumor, destabilizated non-tumor and tumor cells *in vitro* according to its influence on the MGMT and MARP expression levels. Elderberry bark lectin SNA-I had only slight effect on MARP expression in stable non-tumor cell line 4BL. But in cell line on the stage of induced secondary genomic instability SNA-I was shown to substantionaly increase MARP expression level. And in malignant cell line Hep-2 we demonstrated both supress and enhance of MARP and MGMT expression levels under the SNA-I treatment.

In early works lectins were assumed to be only carbohydrate-binding proteins, but further they were shown to affect the different kinds of enzyme activities. For the first time we have shown that MARP and repair enzyme MGMT expression can be regulated by these biologically active substances as well.

Conclusions

Lectins of plant and animal origin (perk egg lectin, lentil seeds lectin and elderberry bark lectin) are able to modulate the expression of proteins recognized by anti-MGMT monoclonal antibodies (MGMT and MARP).

The influence of SNA-I on MARP and MGMT expression levels depends on origin and genomic stability of cell line. Destabilized 4BL cell line was shown to be more sensitive to SNA-I modulating action. In malignant cells both expression up- and downregulating effect of SNA-I lectin was described, so SNA-I is perspective for futrther study as potential drug in anti-tumor therapy optimization schemes.

References

1. Pegg A.E. Multifaceted Roles of Alkyltransferase and Related Proteins In DNA Repair, DNA Damage, Resistance to Chemotherapy and Research Tools // Chem Res Toxicol. – 2011. – 24 (5). – P. 618–639.
2. Gerson S.L. MGMT: its role in cancer aetiology and cancer therapeutics // Nat Rev Cancer. – 2004. – 4 (4). – P. 296–307.
3. Pegg, A.E. Repair of O6-Alkylguanine by Alkyltransferases // Mutat. Res. – 2000. – 462. – P. 83–100.
4. Lukash L.L., Man'ko V.G., Lylo V.V. Role of O6-Alkylguanine-DNA alkyltransferase in repairing lesions, induced by alkylating compounds // Biopolym. Cell. – 2001. – 17 (4). – P. 265–277.
5. Christmann M., Kaina B. Transcriptional regulation of human DNA repair genes following genotoxic stress: trigger mechanisms, inducible responses and genotoxic adaptation // Nucl Acids Res. – 2013. – 41 (18). – P. 1–18.
6. Gerson S.L. MGMT: its role in cancer aetiology and cancer therapeutics // Nat. Rev. Cancer. – 2004. – 4 (4). – P. 296–307.
7. Mitra S. MGMT: a personal perspective // DNA Rep. – 2007. – 6 (8). – P. 1064–1070.
8. Kyrtopoulos S.A. O6-Alkylguanine-DNA alkyltransferase: influence of susceptibility to the genetic effects of alkylating agents // Toxic. Lett. – 1998. – 102–103. – P. 53–57.
9. Lylo V.V., Matsevich L.L., Kotsarenko E.V., Babenko L.A., Kornelyuk A.I., Sukhorada E.M., Lukash L.L. Activation of gene expression of the O6-Methylguanine-DNA transferase repair enzyme upon the influence of EMAP II cytokine in human cells *in vitro* // Cytology and Genetics. – 2011. – 45 (6). – P. 373–378.
10. Kotsarenko K.V., Lylo V.V., Macewicz L.L., Babenko L.A., Kornelyuk A.I., Ruban T.A., Lukash L.L. Change in the MGMT Gene expression under the influence of exogenous cytokines in human cells *in vitro* // Cytology and Genetics. – 2013. – 47 (4). – P. 202–209.
11. Kotsarenko K.V., Lylo V.V., Macewicz L.L., Ruban T.A., Luchakivska Yu.S., Kuchuk M.V., Lukash L.L. Influence of some biologically active substances on the MGMT and MARP genes expression in human cells *in vitro* // Cythology and Genetics. – 2014, in press.
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. – 2007. – 6. – P. 1079–1099.
13. Natsume A., Ishii D., Wakabayashi T., Tsuno T., Hatano H., Mizuno M., Yoshida. J. IFN-B down-regulates the expression of DNA repair gene MGMT and sensitizes resistant glioma cells to temozolomide // Cancer Res. – 2005. – 65 (17). – P. 7573–7579.
14. Lavon I., Fuchs D., Zrihan D., Efroni G., Zelikovitch B., Fellig Y. Siegal T. Novel mechanism whereby nuclear factor kappaB mediates DNA damage repair through regulation of O(6)-methylguanine-DNA-methyltransferase // Cancer Res. – 2007. – 67 (18). – P. 8952–8959.
15. Lylo V.V., Macewicz L.L., Kotsarenko K.V., Babenko L.A., Kornelyuk A.I., Suhorada E.M., Lukash L.L. Induction of repair enzyme O^6methylguanine DNA methyltransferase gene expression under the influence of cytokine EMAP II in human cells *in vitro* // Cytology and Genetics. – 2011. – 45 (6). – P. 373–378.
16. Lis H., Sharon N.. Lectins: carbohidrate specific proteins that mediate cellular recognition // Chem. Rev. – 1998. – 98. – P. 637–674.
17. Karpova I.S., Koretskaya N.V. Study on modifying action of lectins on the toxic and mutagenic effects of Ni(II) ions in Bacillus subtilis culture // Biopol Cell. – 2003. – 19 (3). – P. 224–230.
18. Lukash L.L., Karpova I.S., Miroshnichenko O.S., Tikhonova T.N., Lylo V.V., Man'ko V.G., Sukhorada E.M., Golynskaia E.L. The effect of the lectin from *Sambucus nigra* inflorescences on spontaneous and alkylating agent-induced mutagenesis in mammalian somatic cells // Cytol. Genet. – 1997. – 31 (5). – P. 52–60.
19. Matsevych L.L., Kovalenko O.O., Suhorada O.M., Lukash L.L. The influence of proteins on the genetic variability of cell populations *in vitro* // Factors of experimental evolution of organisms (Collected works under the editor of Acad. M.V. Royik). – Kyiv: Agricultural Sciences, 2003. – P. 91–97.
20. Kovalenko O.O., Kostetskaia K.V., Lukash L.L. The influence of proteins on the genetic variability of cell populations *in vitro* // Biopolym. Cell. – 2006. – 22 (1). – P. 33–38.
21. Macewicz L.L., Suchorada O.M., Lukash L.L. Influence of Sambucus nigra bark lectin on cell DNA under different *in vitro* conditions // Cell Biology International. – 2005. – 29. – P. 29–32.
22. Lylo V.V., Piven' O.O., Serebriakova K.V., Matsevich L.L., Lukash L.L. The influence of lectins on some repair processes in mammalian cells *in vitro* // Ukr Biochem. J. – 2008. – 80 (6). – P. 60–65.
23. Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature. – 1970. – 227. – P. 680–685.
24. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // Anal. Biochem. – 1976. – 72. – P. 248–254.
25. Aldridge G.M., Podrebarac D.M., Greenough W.T., Weiler I.J. The use of total protein stains as loading controls: An alternative to high-abundance single- protein controls in semi-quantitative immunoblotting // Journal of Neuroscience Methods. – 2008. – 172 (2). – P. 250–254.
26. Macewicz L.L., Kushniruk V.O., Iatsyshyna A.P., Kotsarenko K.V., Lylo V.V., Akopyan G.R., Huleuk N.L., Mykytenko D.M., Lukash L.L. Correlation the level of mutagenesis with expression of reparative enzyme O6-

metylhuanin DNA methyltransferase during establishment of cell lines *in vitro* // Biopol Cell. – 2013. – 29, N 6. – P. 485–492.

MACEWICZ L.L., LYLO V.V., KARPOVA I.S., KOTSARENKO K.V., RUBAN T.A., LUKASH L.L.
Institute of Molecular Biology and Genetics of Natl. Acad. Sci. of Ukraine,
Ukraine, 03680, Kyiv, Akad. Zabolotnogo str., 150, e-mail: lukash@imbg.org.ua

PLANT AND ANIMAL LECTINES AS MODULATORS OF *MGMT* AND *MARP* GENE EXPRESSION *IN VITRO*

Aims. Previously for the first time we have studied the ability of lectins to influence the processes of mutagenesis and antimutagenesis in different test systems. The aim of present study was to examine the effect of panel of lectins on the MGMT and MARP expression levels in tumor and non-tumor mammalian cells *in vitro*. ***Methods***. Standard cell cultivation methods and Western blot analysis were used. ***Results***. The influence of plant and animal lectins (perk egg lectin, lentil seeds lectin and elderberry bark lectin) on expressiom of proteins recognized by anti-MGMT monoclonal antibodies (MGMT and MARP) on stable and destabilized human non-tumor and tumor-derived cell lines was studied. ***Conclusions***. Studied lectins are able to modulate the expression of MGMT and MARP. The influence of SNA-I on MARP and MGMT expression levels depends on origin and genomic stability of cell line. SNA-I is perspective for further study as potential drug in anti-tumor therapy optimization schemes.
Key words: MGMT expression, MARP expression, lectins, NiCl2, cell lines.

UDC 633.11:631.523:633.11
MOTSNY I.I. [1], SUDARCHUK L.V. [1], GALAEV A.V. [1], CHEBOTAR S.V. [1, 2]
[1] *Plant Breeding and Genetics Institute – National Center of Seed and Cultivar investigations,*
Ukraine, 65036, Odessa, Ovidiopolskaya dor., 3, e-mail: motsnyyii@gmail.com
[2] *Odessa National Mechnikov University, Department of Genetics and Molecular Biology,*
Ukraine, 65026, Odessa, Dvoryanskaya str., 2

APPLICATION OF PCR MARKERS FOR DETECTING $1B_L.1R_S$ WHEAT-RYE CHROMOSOME TRANSLOCATIONS AND (1B)1R SUBSTITUTIONS

Nikolai Vavilov was the first to recognize the utilization of wheat relatives is a promising source for wheat improvement [1]. As an development of Vavilov`s ideas a number of wheat introgression stocks with a high resistance to powdery mildew, leaf and stem rusts, frost tolerance, high protein content and some morphological characters has been obtained as a result of wide crosses [2, 3]. For a successful practical application the stocks require an identification of the alien introgressions. DNA markers become a useful tool for gene or chromosome identification, especially being valuable in respect of new for wheat an alien genetic material.

This paper deals with PCR marker assisted detection of (1B)1R wheat-rye chromosome substitution and $1B_L.1R_S$ translocation, their meiotic behavior and genetic analysis of certain alien characters, incorporated into wheat. The investigation was carried out within a program for the development of a genetic collection of bread wheat lines with qualitative characters.

Material and methods

A set of original primitive introgression stocks ($2n$ = 42): Erythrospermum 200_97-2 (in further E200_97-2), Erythrospermum 217_97 (E217_97), Hostianum 242_97-1 (H242_97-1), Hostianum 242_97-2 (H242_97-2), Hostianum 273_97 (H273_97), Hostianum 274_97 (H274_97) and OH232_03, collection sib-strains H74_90-245 and H74_90-258, winter bread wheat cv. Odesskaya 267 (Od267) and F_1 hybrids between Od267 and all the lines have been investigated. The majority of the stocks were developed from a cross: triticale (8x) cv. AD825/*T. durum* Desf. cv. Chernomor and spontaneous hybridization of the F_3 hybrids with the strain H74_90-245 or H74_90-258, or without it. Triticale AD825 is a primary amphidiploid (*T. aestivum* L. cv. Hostianum 237/*S. cereale* L. cv. Voronezhskaya SHI) [4]. The strains H74_90-245 and H74_90-258 were derived in Dobroudja Agricultural Institute (General Toshevo, Bulgaria) from the step cross: Dr. Savov`s synthetic (*T. timopheevii* Zhuk./*Ae. tauschii* Coss.)/Tom Pouce