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ALTERATIONS OF RAT BRAIN CHROMATIN PHOSPHOLIPIDS CONTENT UNDER THE SEPARATE AND JOINT ACTION OF CISPLATIN AND ESTRADIOL

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The alterations in total amount of phospholipids and their individual fractions content in chromatin preparations from female rat brain cells after the estradiol and cisplatin separate and joint action were investigated. It was revealed that in case of separate action these drugs exhibit opposite effects on total quantity of phospholipids as well as on absolute amount of individual phospholipid fractions. Cisplatin and estradiol joint action restored the baseline value of chromatin phospholipids total amount and had different impact on the content of its individual fractions. These quantitative alterations of rat brain chromatin phospholipids may be helpful for reducing cisplatin toxicity.

Cisplatin – estradiol – rat brain chromatin – phospholipids

Հետազոտվել է էգ առնետների գլխուղեղի քրոմատինային ֆոսֆոլիպիդների ընդհանուր պարունակության և դրանց առանձին ֆրակցիաների բացարձակ քանակների փոփոխությունները ցիսպլատինի և էստրադիոլի առանձին և համատեղ ազդեցությունից հետո: Պարզվել է, որ առանձին կիրառելու դեպքում այս դեղերը դրսևորում են իրենց բնորոշ ազդեցությունները ինչպես քրոմատինային ֆոսֆոլիպիդների ընդհանուր քանակի, այնպես էլ դրանց առանձին ֆրացիաների բացարձակ պարունակության վրա: Ցիսպլատինի և էստրադիոլի համատեղ կիրառումը վերականգնում է ֆոսֆոլիպիդների ընդհանուր քանակի ստուգիչ տարբերակի արժեքները և տարբեր ազդեցություն է թողնում ֆոսֆոլիպիդների առանձին ֆրակցիաների բացարձակ քանակների վրա: Առնետի գլխուղեղի քրոմատինային ֆոսֆոլիպիդների քանակական փոփոխությունները կարող են կարևոր լինել ցիսպլատինի տոքսիկության և անցանկալի կողմնային ազդեցությունների նվազեցման առումով:

Ցիսպլատին – էստրադիոլ – առնետի գլխուղեղի քրոմատին – ֆոսֆոլիպիդներ

Исследовалось содержание общих фосфолипидов и их индивидуальных фракций в препаратах хроматина клеток головного мозга крыс самок при отдельном и совместном применении цисплатина и эстрадиола. Показано, что при отдельном применении эти лекарства проявляют присущие им воздействия как на количество тотальных фосфолипидов хроматина, так и на содержание отдельных фосфолипидных фракций. Совместное применение эстрадиола и цисплатина восстанавливает контрольный уровень тотального количества фосфолипидов хроматина и по разному влияет на содержание отдельных фосфолипидных фракций. Выявленные изменения количества хроматиновых фосфолипидов по всей вероятности, могут быть связаны со снижением токсичности цисплатина и смягчением нежелательных побочных эффектов, вызванных им.

Цисплатин – эстрадиол – хроматин головного мозга крыс – фосфолипиды

Chromatin is a macromolecular complex composed of distinct molecules such as DNA, proteins and certain amount of lipids [18]. These components are maintaining the structure of chromatin and ensure its proper functioning. It is well known, that chromatin state defines the functional genomic regulation, including activation or repression of transcription, DNA replication, chromatin condensation etc. These processes are regulated by covalent modifications of chromatin components, including DNA methylation and various modifications of histones, like methylation, acetylation, phosphorylation and other modifications [18]. Generally, transcriptionally active or euchromatic DNA regions are hypomethylated and are associated with hyper acetylated histones, whereas inactive or heterochromatin contains hypermethylated DNA and deacetylated and hypomethylated histones [18].

Nuclear phospholipids are involved in such processes as acetylation or methylation [5, 7, 21]. Nuclear lipids modulate histone modifications by changing the activity of enzymes catalyzing these modifications and thereby participate in gene regulation [6, 16, 21]. Nuclear lipids don't just modified or bind to histones, they can also directly bind to DNA molecule. Lipids could directly affect DNA supercoiling, which is important for transcription, replication, and recombination/repair. Tightly bound to DNA lipids may represent a new information level or 'lipid code" in genomic DNA [6, 7, 16, 21]. The exact mechanism of how chromatin bound lipids, may influence on state of chromatin is not yet known, but it is possible that lipid quantitative changes may contribute to this [1, 8, 10, 20].

Nowadays it is well known that cisplatin (cis-diaminedichloroplatinum II) is an effective antitumor drug, which is widely used in chemotherapeutic practice. DNA is considered as the primary target for cisplatin [2, 5, 17]. On the other hand it is well known also that nuclear receptors of steroids are transcription factors and can interact with chromatin [9, 13, 14]. Genomic effects of steroids involving migration of the dimerised estrogen-receptor complexes to the cell nucleus, and direct interaction with chromatin at specific DNA sequences known as estrogen response elements (EREs) [9, 13, 14]. Consequently chromatin and its certain components could be the potential sites of these interactions. It cannot be excluded involvement of chromatin associated lipids in molecular mechanisms of cisplatin and estradiol action. We postulate that it is possible implementation of cisplatin and estradiol effects realization via quantity alterations of chromatin phospholipids [10, 11].

In light of the foregoing it seems interesting to explore the cisplatin and estradiol separate and joint *in vivo* action on phospholipids content of chromatin preparations from rat brain cells.

In this paper the alterations of quantities of total phospholipids as well as changes in their individual fractions content in chromatin preparations from rat brain cells after cisplatin and estradiol separate and its joint *in vivo* action were investigated.

Materials and methods. The investigation was performed on adult female albino rats (120-150 g weight). The animals were kept in 20-22^{0}C and 12 hours dark/light conditions with free access to water and food (animals were fed with commercial rat feed) in the animal house of faculty of biology of Yerevan state university.

Experiences were carried out according to the "International Recommendations on Carrying out of Biomedical Researches with use of Animals" (CIOMS, 1985), to the "Human Rights and Biomedicine the Oviedo Convention" (CE, 1997), to the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005) and approved by the National Center of Bioethics (Armenia).

The animals were divided into 4 groups. The group 1 was a control group of animals without treatment. Animals of group 2 and group 4 received a single dose of cisplatin (8 mg/kg). Cisplatin was injected peritoneal. Exposition time for cisplatin was 24 hours. The group 3 was

treated with estradiol (200 mcg/kg, injected peritoneal). Exposition time for estradiol was 4 hours. Animals from the group 4 were received the same single dose of estradiol within 20 hours after the cisplatin injection (4 hours before decapitation). All animals were killed by decapitation through appropriate time after the anesthesia with chloroform. Then, animals were sacrificed, and the brain tissue was extracted from each group of animals and used for isolation of nuclei by the method of Blobel and Potter [4].

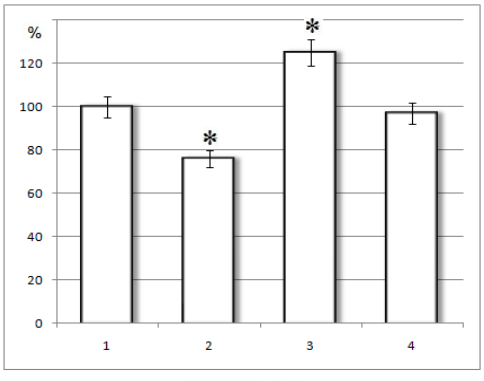
The preparations of pure nuclei (all 4 variants) were used for the isolation of chromatin [19]. Phospholipids of chromatin fraction from rat brain cells were extracted by Bligh and Dayer [3]. The fractionation of phospholipids was performed by micro thin layer chromatography (microTLC) using 6x9 sm^2 plates with silicagel L and chloroform – methanol – water in ratio 65:25:4 as a dividing mixture. After the chromatography the plates were dried up at room temperature and were treated by 15.6 % $CuSO_4$ in 8 % phosphoric acid. Then the elaborated plates were heated at 180^0C for 15 minutes. The quantitative estimation of separated and specific died phospholipids was carried out by special computer software Fujifilm Science Lab 2001 Image Gauge V42, which was designed for densitometry. Obtained results were treated by statistics and expressed as M±m from 6 independent experiments. Statistical differences in the results between groups were evaluated by the Student's t-test.

Results and Discussion. Cisplatin *in vivo* action reliably decreases the total amounts of phospholipids of chromatin preparations from rat brain cells by 24% [10]. At the same time the steroid hormone estradiol increases the quantity of total phospholipids in observed preparation up to 25%. However the joint action of cisplatin and estradiol restored the baseline value of phospholipids total amount of chromatin preparations from female rat brain. (tab. 1, fig.1).

Table 1. Total phospholipids content (in mcg/g of tissue) in nuclear preparations of rat brain Cells in baseline and after the cisplatin and estradiol separate and joint in vivo action

*-p<0,05

Variants	**Phospholipids content mcg/g tissue**
Baseline	184,00±6,70
Cisplatin	*140,00±3,65
Estradiol	*230,00±4,00
Cisplatin+ Estradiol	178,00±5,64

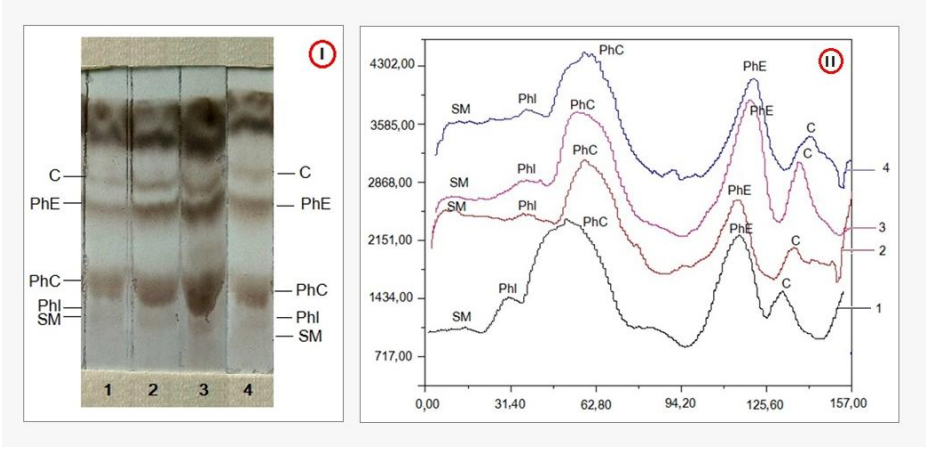


* - P < 0,05

Fig.1. Changes of total phospholipids content in nuclear preparation of rat brain cells after the cisplatin and estradiol separate and joint treatment.
1 – baseline, 2 – after the cisplatin separate action [10], 3 – after the estradiol separate action, 4 – after joint action of cisplatin and estradiol

The results have shown that in case of separate action cisplatin and estradiol exhibit opposite effects on total amount of chromatin phospholipids, whereas in case of their joint action steroid neutralizes the suppressor effect of cisplatin (tab. 1, fig.1).

The fractionation of chromatin phospholipids by the microTLC method revealed five individual phospholipids in baseline as well as after the cisplatin and estradiol separate and joint action. (fig.2). Neither the separate nor the combination action of these drugs does not cause qualitative changes of phospholipids (fig.2). Sphingomyelin, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and cardiolipin were obtained among the phospholipids of rat brain cells chromatin preparations (fig.2).



A B

Fig.2A. Phospholipids content in nuclear preparation of rat brain cells after the cisplatin and estradiol separate and joint treatment.
Fig. 2B. Chromatograms (I) and dencitograms (II) of fractionated by microTLC chromatin phospholipids from rat brain nuclei
1 – baseline; 2 – after the cisplatin separate action [10]; 3 –after the separate action of estradiol; 4 – after the cisplatin and estradiol joint action.
SM – sphingomyelin, PhI – phosphatidylinositol, PhC – phosphatidylcholine, PhE – phosphatidylethanolamine, C – cardiolipin.

The computer program Fujifilm Science Lab 2001 Image Gauge V42 allows quantifying separated by microTLC phospholipids. The results are shown in the table 2 (tab. 2). Phosphatidylethanolamine and phosphatidylcholine were the major components and formed about 58% of total phospholipids in all observed chromatin preparations of rat brain, The relative content of sphingomyelin, phosphatidylinositol and cardiolipin was correspondingly 12,4 %, 11,42 % and 18,54 % (tab. 2).

The most appreciable changes among the minor phospholipid fractions were observed in case of cardiolipin (the difference was about 6 % after the estradiol separate and joint with cisplatin action) (tab. 2). It is obvious that the obtained changes in percentage content do not represent the reality of alteration in real content of phospholipid individual fractions after the drugs action.

In order to clear up this problem the absolute quantities of individual phospholipids (in micrograms per gram of brain tissue) in all observed chromatin preparations before and after the drugs injection were determined (tab. 3). The results showed the reliable changes in the absolute quantity of all individual phospholipids after the cisplatin and estradiol

separate injection. In case of cisplatin separate *in vivo* action the quantities of all five phospholipids were diminished, in different degrees (tab. 3) [10].

Table 2. The relative content (in percentage) of individual fractions of phospholipids in nuclear preparations of rat brain cells in baseline and after the cisplatin and estradiol separate and joint action

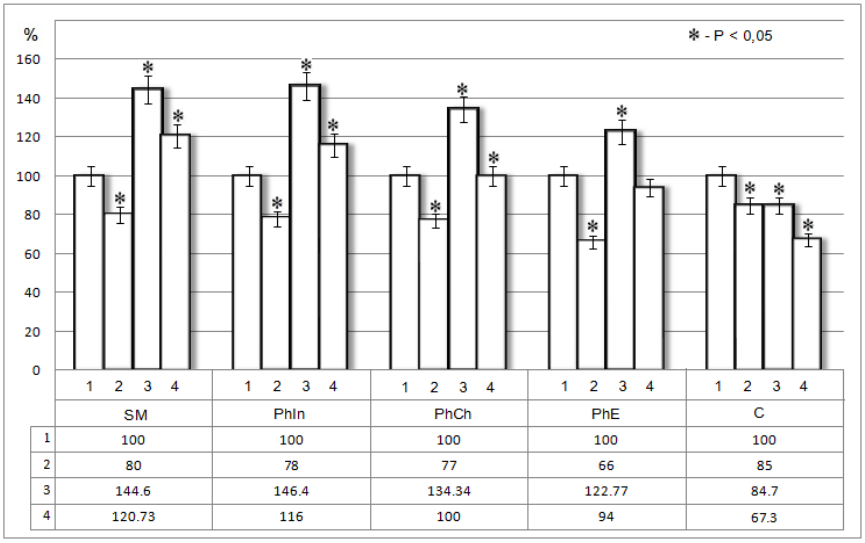
N	Phospholipids	Baseline	Cisplatin Injection [10]	Estradiol injection	Cisplatin and estradiol joint injection
1	Sphingomyelin	12,40 ± 0,60	13,00 ± 0,35	14,40±0,54	15,48±0,65
2	Phosphatidylinositol	11,42 ± 0,64	11,67 ± 0,42	13,36±0,34	13,67±0,86
3	Phosphatidylcholine	33,30 ± 1,08	33,68 ± 0,64	35,85±0,75	34,33±1,04
4	Phosphatidylethanolamine	24,34 ± 1,00	21,00 ± 0,84	23,87±0,43	23,67±0,80
5	Cardiolipin	18,54 ± 1,00	20,65 ± 0,32	12,52±0,45	12,85±0,60
Total content		100	100	100	100

On the contrary estradiol separate injection increased the amount of 4 out of 5 phospholipids The exception is cardiolipin the absolute quantity of which was reduced after the steroid separate treatment (tab. 3, fig.3). Estradiol separate treatment leads to an increase in absolute content of sphingomyelin by 45 %, phosphatidylinositol by 46 %, phosphatidylcholine 34 % and phosphatidylethanolamine 23 % (tab. 3 and fig.3). Along with these changes, there has been a decline of cardiolipin content by 15 % as compared to baseline (tab. 3, fig.3).

Cisplatin and estradiol joint action manifested the tendency of restoring the baseline quantity of phosphatidylcholine and phosphatidylethanolamine, whereas the amount of sphingomyelin and phosphatidylinositol was increased (respectively by 21 % and 16 %) and the quantity of cardiolipin was decreased by about 33 % after the joint action of these drugs (tab. 3, fig.3).

Table 3. The absolute quantities (in micrograms per gram of tissue) of individual Phospholipid fractions in nuclear preparations of female rat brain cells in baseline and after the cisplatin and estradiol separate and joint action.
*-p<0,05; **-p<0,10

N	Phospholipids	Baseline	Cisplatin Injection [10]	Estradiol injection	Cisplatin and estradiol joint injection
1	Sphingomyelin	22,82 ± 1,10	*18,20 ± 0,50	*33,00±1,24	27,55±1,14
2	Phosphatidylinositol	21,00 ± 1,20	*16,35 ± 0,60	*30,74±0,78	**24,35±0,86
3	Phosphatidylcholine	61,38 ± 1,90	*47,15 ± 0,90	*82,46±1,73	61,10±1,36
4	Phosphatidylethanolamine	44,80 ± 1,85	*29,40 ± 0,84	*55,00±1,10	42,13±1,80
5	Cardiolipin	34,00 ± 1,62	*28,90 ± 0,32	**28,80±0,92	*22,87±1,07



	SM	PhIn	PhCh	PhE	C
1	100	100	100	100	100
2	80	78	77	66	85
3	144.6	146.4	134.34	122.77	84.7
4	120.73	116	100	94	67.3

Fig. 3. The alteration (in percent) in individual phospholipid quantities in rat brain chromatin preparations after the cisplatin and estradiol separate and joint action.
1 – baseline, 2 – after the cisplatin separate action [10], 3 – after the estradiol separate action, 4 – after joint action of cisplatin and estradiol
SM – sphingomyelin, PhI – phosphatidylinositol, PhC – phosphatidylcholine, PhE – phosphatidylethanolamine, C – cardiolipin

Thus co-use of drugs with opposite effects actually restores the control level of neutral phospholipids (phosphatidylcholine and phosphatidylethanolamine) and manifests itself in different ways for acidic lipids (sphingomyelin, phosphatidylinositol and cardiolipin) (tab. 3, fig.3).

These results revealed the antagonistic nature of the effects of cisplatin and estradiol in relation to phospholipid metabolism in nuclear fractions of rat brain cells. Since lipid metabolism participates in the regulation of many cellular processes such as cell growth, proliferation, differentiation, apoptosis, chemotherapy response and drug resistance etc. [1, 6, 16], our results take on considerable importance.

It is well known that chromatin phospholipids in dose dependent manner *are capable of* regulating DNA replication, transcription and gene expression [1, 5, 16, 21]. Recent advances demonstrated the involvement of nuclear lipids in remodeling of chromatin and epigenetic regulation of gene expression [5, 16, 21]. In light of the fact that cell nuclei is a site of lipids active metabolism, one may conclude that these remarkable changes in absolute quantities of individual phospholipid fractions may be the consequence of investigated medications on lipid metabolic pathways in nuclei. These changes in turn may offer some serious prerequisites for alteration the functioning those processes where these phospholipids participated. It is known that phosphatidylethanolamine promotes the decondensation of chromatin, induces transition of chromatin from solenoid to nucleosome [16]. Likewise, alteration of DNA-bound cardiolipin quantity may effect on regulation of activities of DNA topoisomerase, RNA polymerase, DNA replication as well as alters the condensation of chromatin [5, 6, 16, 21].

It is known also that the chromatin structure could be regulated by phosphoinositides via binding to the C-terminal tail of histone H1 [5, 8].Considering the fact that sphingomyelin and phosphatidylinositol are the members of corresponding nuclear signaling systems one can imagine the consequences of quantitative changes of

these phospholipids [1, 5, 16, 21].

So, changes in absolute quantities of phospholipid fractions in chromatin caused by cisplatin and estradiol separate action can mediate the own specific effects of these drugs. It's been proven that cisplatin causes undesirable side effects, which manifest in various intoxications, including nephrotoxicity, ototoxicity, gastro toxicity, myelosuppression, allergic reactions [2, 15, 17]. It was demonstrated that steroid hormones such as estradiol and progesterone has a dose dependent effect on reducing cisplatin induced nephrotoxicity [12].

It can be assumed that the revealed alterations in absolute quantities of chromatin phospholipids separate fractions in case of joint action of cisplatin and estradiol have contributed to extenuating of cisplatin toxic side effects by steroid hormone.

Cisplatin and estradiol separate action as well as its joint injection are capable to provoke deep metabolic changes in brain cells. Alterations of chromatin phospholipids absolute quantity are tools by which specific properties of cisplatin and estradiol are represented in case of their separate action and revealed peculiar summation of its antagonistic effects in case of joint action of these drugs.

All these effects confirm the supposition that alteration of phospholipids quantity in chromatin of brain cells can effect on basic functions of nuclei: replication and transcription.

REFERENCES

1. *Albi E.* Role of Intranuclear Lipids in Health and Disease. Clinical Lipidology, *6*, 1, 59-69, 2011.
2. *Aldossary S.A.* Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. Biomedical & Pharmacology Journal., *12*, 1, 7-15, 2019.
3. *Bligh E.G., Dyer W.Y.* A Rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., *37*, 911-917, 1959.
4. *Blobel G., Potter V.R.* Nuclei from Rat Liver: Isolation Method That Combines Purity with High Yield, Science, *154*, 76-79, 1966.
5. *Castano E., Yildirim S., Fáberová V., Krausová A., Uli˘cná L., Papr˘ cková D., Sztacho M., Hozák P.* Nuclear Phosphoinositides - Versatile Regulators of Genome Functions. Cells, 8, *649*, 1-19, 2019. Doi:10.3390/cells8070649.
6. *Conte C., Codini M., Albi E.* Chromatin Lipid Microdomains. Int J Pathol Clin Res, *4*, 2, 1-3, 2018. doi: 10.23937/2469-5807/1510075
7. *Elouarrat D.* in book "Linking lipids to acetylation", Chapter 3, Nuclear phospholipids as epigenetic regulators, 47-61, (127p), 2013.
8. *Fiume R., Faenza I., Sheth B., Poli A.,. Vidalle M.C, Mazzetti C., Abdul S.H., Campagnoli F., Fabbrini M., Kimber S.T., Mariani G.A., Xian J., Marvi M.V., Mongiorgi S., Shah Z., Divecha N.* Nuclear Phosphoinositides: Their Regulation and Roles in Nuclear Functions. Int. J. Mol. Sci., *20*, 2991, 1-20, 2019. doi:10.3390/ijms20122991.
9. *Fuentes N., Silveyra P.* Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol., *116*, 135-170, 2019. doi:10.1016/bs.apcsb.2019.01.001.
10. *Gevorgyan E.S., Yavroyan Zh.V., Hakobyan N.R., Hovhannisyan A.G.* Cisplatin in vivo influence on lipid content of chromatin on rat brain cells. Proceedings of the Yerevan state university, Chemistry and Biology, 51, 1, 21–26, 2017.
11. *Gevorgyan E.S., Yavroyan Zh.V., Hakobyan N.R., Hovhannisyan A.G., Sargsyan E.G.* Cisplatin and estradiol joint action on phospholipid composition of nuclear fraction from female rat brain cells. Biolog. Journal of Armenia, 3, *71*, 67-72, 2019.
12. *Ghasemi M., Nematbakhsh M., Pezeshki Z., Soltani N., Moeini M., Talebi A.* Nephroprotective effect of estrogen and progesterone combination on cisplatin‐induced nephrotoxicity in ovariectomized female rats. Indian Journal of Nephrology, 26, 3, 167-175, 2016.

13. *Hamilton K. J., Hewitt S.C., Arao Y., Korach K.S.* Estrogen Hormone Biology Curr. Top Dev. Biol., *125*, 109-146, 2017. doi:10.1016/bs.ctdb.2016.12.005.
14. *Hua H., Zhang H., Kong Q., Jiang Y.* Mechanisms for estrogen receptor expression in human cancer. Exp Hematol Oncol, 7, *24*, 1-11, 2018.
15. *Jadon A.S., Bhadauriya P., Sharma M.* An integrative review of Cisplatin: the first metal Anti-Tumor drug. Journal of Drug Delivery and Therapeutics.; *9*, 3, 673-677, 2019.
16. *Kuvichkin V.* The role of interactions of nucleic acids with lipids in nuclear pore assembly, genome expression and carcinogenesis. MOJ Proteomics Bioinformatics., *7*, 4, 215-217, 2018.
17. *Makovec T.* Cisplatin and beyond: molecular mechanisms of action and drug resistance development in cancer chemotherapy. Radiol. Oncol., *53*, 2, 148-158, 2019. doi: 10.2478/raon-2019-0018.
18. *Suganuma T., Workman J.L.* Chromatin and Metabolism Annu. Rev. Biochem., *87*, 27-49, 2018.
19. *Umansky S.R., Kovalev J.J., Tokarskaya V.Y.* Specific Interaction of Chromatin Non-Histone Proteins with DNA. Biochem. Biophys. Acta, *383*, 3, 242-248, 1975.
20. *Zaina S., Dossing K.B.V., Lindholm M.W., Lund G.* Chromatin modification by lipids and lipoprotein components: an initiating event in atherogenesis? Current Opinion In Lipidology.. *16*, 5, 549-553, 2005.
21. *Zhdanov R.I., Schirmer E.C., Venkatasubramani A.V., Kerr A.R.W., Rodriges Blanco G.,Kagansky* A. Lipid Contribute to Epigenetic Control via Chromatin Structure and Functions, Science Open Res., *10*, 15, 1-9, 2015.

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