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RESEARCH ARTICLE

Evaluation of Changes in the Expression Profile of mRNA and Protein-encoding Adiponectin in Ishikawa Cell Line under the Influence of Cisplatin – Preliminary Report

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Abstract: ***Background*:** A reduced concentration of adiponectin is considered as an independent factor of the risk of inducing endometrial cancer. Cisplatin is a drug used in the therapy of this type of neoplasm. However, knowledge of the effects of cisplatin on the adiponectin level is still limited.

***Objective*:** The purpose of this study was to assess the impact of cisplatin depending on the concentration and time of exposition of the cells to the drug on the adiponectin level in the endometrial cancer cell line.

***Methods*:** Cells of endometrial cancer cell line Ishikawa were exposed for 12,24 and 48 hour periods to cisplatin with the following concentrations: 2.5μM, 5μM, 10μM. The changes in the expression profile of adiponectin were compared to the RtqPCR reaction and ELISA test. The STATISTICA 13.0 PL program was used for statistical analysis ($p<0.05$).

***Results*:** In the culture without the drug, the concentration of adiponectin was statistically lower than in the cell culture incubated with the drug. Changes on the mRNA level seem to be more specific than on the protein level, although in both cases, the same trend in the expression changes was noted.

Discussion: The longer the time of exposition of the cells to the drug, the expression of mRNA, and the adiponectin protein increased. Changes in the expression profile were characterized statistically ($p<0.05$).

***Conclusion*:** Cisplatin, in a noticeable way, changes the expression profile of adiponectin. Molecular analysis indicated that in the case of endometrial cancer therapy should be implemented with a concentration of no less than 5 μM.

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1. INTRODUCTION

Adiponectin takes part in the metabolism of glucose and lipids. It reveals many favorable effects, including a better sensitivity to insulin, stimulation of endothelial synthase of nitric oxide and most importantly, anti-inflammation activity. It plays a protective role against Non-Alcoholic Fatty Liver Disease (NAFLD) or diabetes, among others [1]. Adiponectin activity shows anti-inflammatory characteristics through antagonizing the Tumor Necrosis Factor-alpha (TNF-α) and interleukin 6 (IL-6) as well as stimulating the anti-inflammatory effect of IL-10 [2].

Discovering adiponectin, as an insulin-sensitizing protein, was connected with the reduction of the mRNA levels for the gene coding for adiponectin in obese people [3, 4].

It was observed that the unlikely secretion of the mentioned protein is closely related to the interference of the metabolism of glucose. Its low concentration may be predisposed to fatten the liver and to cause advanced damage to the liver [5, 6]. One of the signaling pathways activated by adiponectin is the pathway dependant on the protein kinase activated by Adenosine Monophosphate (AMP), or AMPK. This kinase is characterized by the ability to restrain FAS, a

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key enzyme with lipogenic activity, whose abnormal expression profile was noted in the colon, breast, prostate, and ovarian cancer. Other than that, AMPK causes phosphorylation, and additionally, activation of the TSC2 suppressor gene, which halts the transduction of the signal down the mTOR pathway. Abnormalities in the mentioned pathway were noted in the colon, breast, prostate, ovarian, liver, and lung cancer [7, 8].

The induction of AMPK with the use of adiponectin also activates endothelial synthase of NO (eNOS) and increases the production of Nitric Oxide (NO). Activation of eNOS is also dependant on the kinase signaling of Akt(Akt) and its' previous mediator 3-kinase phosphatidylinositol (PI-3K). The role of AMPK and activated by its signaling pathway in the context of angiogenesis is twofold. On the one hand, it promotes angiogenesis *in vitro* in answer to hypoxia, on the other hand, it stops the production of new blood vessels. Because of the fact that adiponectin stimulates the AMPK pathway, with which other signaling cascades are connected, the aforementioned molecular pathways could be potential mechanisms through which adiponectin regulates carcinogenesis [7-11]. Nonetheless, however, according to many authors, our research is the first in which the level of adiponectin will be evaluated solely in the endometrial cancer cell line Ishikawa model [12], and the effect of cisplatin on the expression profile of mRNA and adiponectin protein will also be analyzed. The cells engaged in the secretion of the discussed protein are body fat adipocytes [13, 14], therefore also determining its' expression in endometrial cancer cells confirms that during the neoplastic transformation process, it leads to changes in the cell metabolism [15, 16]. For this reason as well, possibly, that changes on the adiponectin level during carcinogenesis happen not only because of changes in the secretion pattern of this protein by body fat cells that make up the tumor. The research carried out by us in this work is an important starting point for analyzing the expression profile of adiponectin in biopsies of endometrial cancer in *in vivo* conditions.

The sixth in the frequency of occurrence around the world, gynecological cancer is endometrial cancer, in which female patients are overweight or obese [17]. In the case of an advanced neoplastic process, chemotherapy is recommended, with the use of cisplatin, among others [18]. The molecular aspect of the effect of this drug is connected with the induction of degradation of genetic DNA material, damaging of mitochondria, induction of oxidation stress and hypoxia, as well as the effect on the signaling pathways connected to apoptosis [18, 19].

The purpose of the study was to examine the expression of adiponectin on the mRNA and protein levels depending on the concentration of cisplatin and exposure time of endometrial cancer cells to the drug.

2. MATERIALS AND METHODS

2.1. Cell Line

The endometrial cancer cells obtained from the Ishikawa cell line (European Collection of Authenticated Cell Cultures (ECACC 99040201) were exposed to three different concentrations of cisplatin, mainly 2.5 μM, 5 μM, and 10 μM. They were incubated with the drug for 12, 24 and 48-hour periods and compared with untreated cells (a control). In this part of our study, the Minimum Essential Medium (MEM) was used dedicated for this cell line, to which 2 mM glutamine, 1% Non-Essential Amino Acids (NEAA), and 5% Fetal Bovine Serum (FBS) was added in accordance with the manufacturer's protocol. Cells were incubated under conditions of a constant temperature of 37˚C and with a 5% CO2 enriched atmosphere. All reagents were obtained from Sigma Aldrich, St Louis, MO, USA. Cisplatin in individual concentrations was added to the cell culture 24 hours after the cells were seeded in six-well plates.

2.2. Real-Time Quantitative Reverse Transcription Reaction

The molecular analysis in our work has two stages. First, changes in the expression pattern of adiponectin under the influence of cisplatin were evaluated on the mRNA level using the real-time quantitative reverse transcription reaction (RTqPCR) with the use of the SensiFAST SYBR No-ROX One-Step Kit (Bioline, London, UK) according to the manufacture's protocol. In the thermal profile of this reaction, the following stages can be indicated: reverse transcription (45ºC for 10 min); polymerase activation (95ºC for 2 min); 40 cycles including denaturation (95ºC for 5 s); annealing (60ºC for 10 s); and elongation (72ºC for 5 s). As an endogenous control, β- actin (*ACTB)* was used. The oligonucleotide primers sequence for adiponectin was forwards: 5`-GTTTTATTGGTTTTAAGGGAGATAT-3`; reverse: 5`- TCCAATCCCACACTAAATACTAAAC-3`, and for *ACTB* was as follows: forwards:5'-TCACCCACACTGTGCCCATCTACGA-3';Reverse:5'-CAGCGGAACCGCTCATTGCCAATGG-3'.

2.3. Enzyme-Linked Immunosorbent Assay ELISA

The second part of the molecular examination was associated with determining the expression of adiponectin on the protein level by using the ELISA assay with the Adiponectin Human solid-phase sandwich Enzyme-Linked Immunosorbent Assay ELISA Kit (Life Technologies Corporation, Invitrogen, USA; catalog number: KHP0041) used. The ELISA assay consists of the following stages: binding the antigen; adding a detector antibody; then adding IgG HRP, TMB substrate solution; and stopping the solution according to protocol. Next, the plate was read at 450 nm and a standard curve generated to determine what the concentration of adiponectin was in the analyzed samples.

2.4. Statistical Analysis

The licensed version of the Statistica 13.0 PL (StatSoft, Cracow, Poland) was used in the statistical analysis with the use of the analysis of variance ANOVA assay, it was shown that the differences were statistically significant, the posthoc Tukey's test was also conducted ($p<0.05$).

3. RESULTS

Changes in the expression profile of adiponectin both on mRNA and protein level under the influence of cisplatin are shown in Table **1** and (Figs. **1** and **2)**. In Fig. (**1**) differences

Table 1. Variances in the level of adiponectin depending on both concentration and exposure time of the Ishikawa cell line to cisplatin.

The Concentration of Cisplatin [μM]	Time [Hours]	RTqPCR (Copy Number of *Adiponectin*/1μg of Total RNA		ELISA Assay Adiponectin [ng/ml]	
		Mean	Standard Deviation	Mean	Standard Deviation
Control (untreated cells)		7237	277	4.036	0.004
2,5	**12**	19108[a]	1248	4.669	0.001
	24	50185[a,b]	1526	4.776	0.003
	48	87253[a,c,d]	1330	4.801	0.002
5	**12**	367091[a]	9260	12.669[a]	0.001
	24	7348104[a,b]	146955	15.778[a,b]	0.001
	48	11486272[a,c,d]	263472	17.011[a,d]	0.011
10	**12**	54111854[a]	824578	20.007[a]	0.006
	24	273567904[a,b]	11284478	20.098[a]	0.001
	48	148687645[a,c,d]	7556148	21.679[a,d]	0.019

[a] – statistically significant differences in the expression of adiponectin between cells exposed to cisplatin *vs*. control $p<0.05$.

[b] – statistically significant differences in the expression of adiponectin between 12 *vs*. 24 hours exposition of cisplatin $p<0.05$.

[c] – statistically significant differences in the expression of adiponectin between 24 *vs*. 48 hours exposition of cisplatin $p<0.05$.

[d] – statistically significant differences in the expression of adiponectin between 12 *vs*. 48 hours exposition of cisplatin $p<0.05$.

in transcriptional activity of mRNA, adiponectin was shown as a log copy number of Adiponectin/1μg of total RNA due to large differences in the amount of transcript between individual cisplatin concentrations.

The expression of adiponectin was statistically significantly lower in the cells of cell line Ishikawa, which made up the control in our experiment when compared to the culture exposed to cisplatin.

With reference to the used concentration of 2.5 μM, cisplatin can be observed on the mRNA level, and when the time of incubation of the cells with the drug is extended, the number of copies of the gene transcript of adiponectin gradually increases. Other than this, statistical analysis confirms that the observed differences in the adiponectin level between certain incubation times are statistically interchangeable. Additionally, on the protein level, it was found that there is a similar tendency for the expression pattern of adiponectin to change, depending on the exposition time of the cells to the drug with a concentration of 2.5 μM. Nonetheless, it is worth noting that on the protein level when using the smallest of the concentrations of cisplatin in this experiment, changes in the expression of adiponectin between the culture exposed to the drug compared to the control culture were irrelevant. Additionally, between certain times of incubation for the discussed concentration of the drug, no statistical similarities were determined, although a small tendency for the concentration of adiponectin to increase as long as the time of incubation for the endometrial cancer cells with cisplatin is extended.

The utilization of a higher concentration of cisplatin, namely 5 μM, also led to changes in the expression profile of the analyzed protein appearing. It can be determined that the longer the time of exposition of the cells to the drug, the expression of adiponectin mRNA increases ($p<0.05$). In turn, by analyzing the expression of adiponectin on the proteome level, with the concentration of 5 μM of cisplatin, differences in the expression of this protein depending on the duration of action of the drug on the cells are statistically significant($p<0.05$). In a visible and relative way, the concentration of adiponectin had a large increase with the use of the concentration of 5 μM of the drug when compared to the lower concentration of cisplatin, or the control culture. Further increases in the dosage of the chemotherapeutic drug to 10 μM, led to a maintained tendency for the level of adiponectin to increase not only on the mRNA level but also on the protein level, which was already observed with lower concentrations of the drug used to stimulate the cells of the endometrial cancer cell line Ishikawa. However, it should be noted, that for the times of 12 and 24 hours, the level of the adiponectin protein remained on, in principle, the same level, with which the elongation of the incubation period to 48 hours, caused an increase in the concentration of adiponectin by 1.581 ng/ml.

4. DISCUSSION

Considering the fact that endometrial cancer is one of the most commonly found gynecological neoplasms in women in the postmenopausal age [17], it seems reasonable to search for the molecular basis behind the induction and development of this sort of neoplasm. One of the key factors which substantially increases the risk of endometrial cancer developing is overweight or obese. When the Body Mass Index (BMI) $\geq$ 32 kg/m^{2,} then this chance is 4 times higher than in women with a BMI of $\leq$ 23 kg/m^2 [20]. Development in the oncological field, including in gynecological oncology

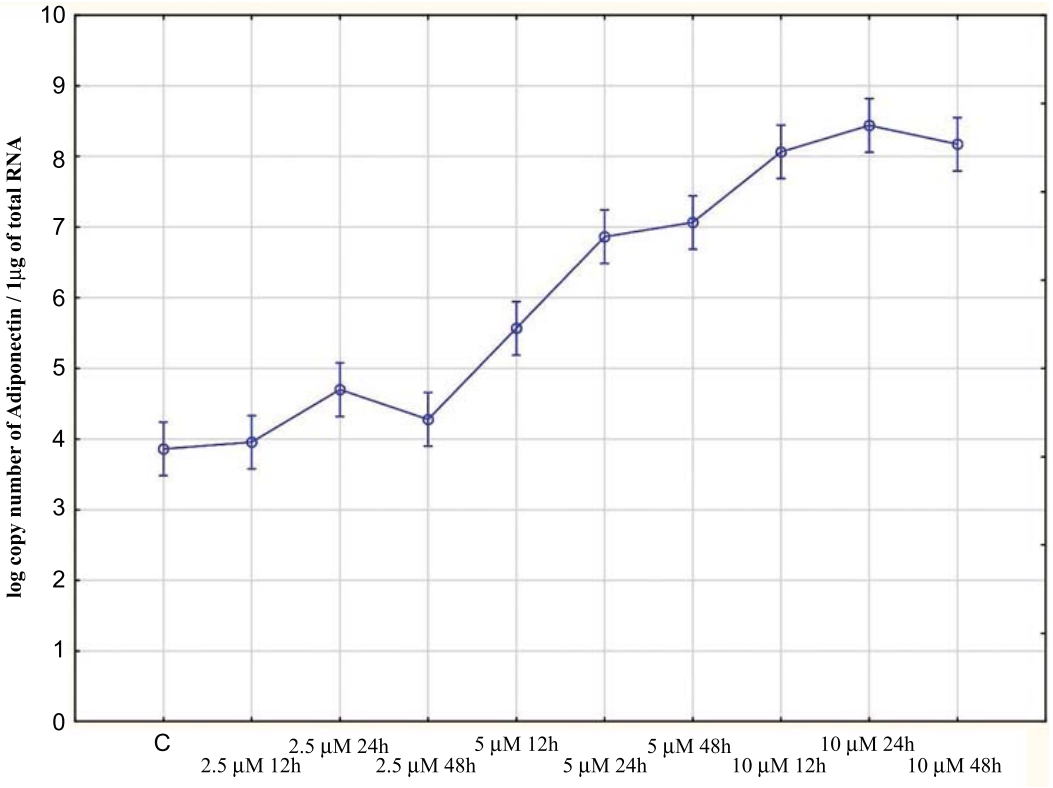

Fig. (1). Changes in the expression profile of mRNA adiponectin depending on concentration and time of exposition of cisplatin. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

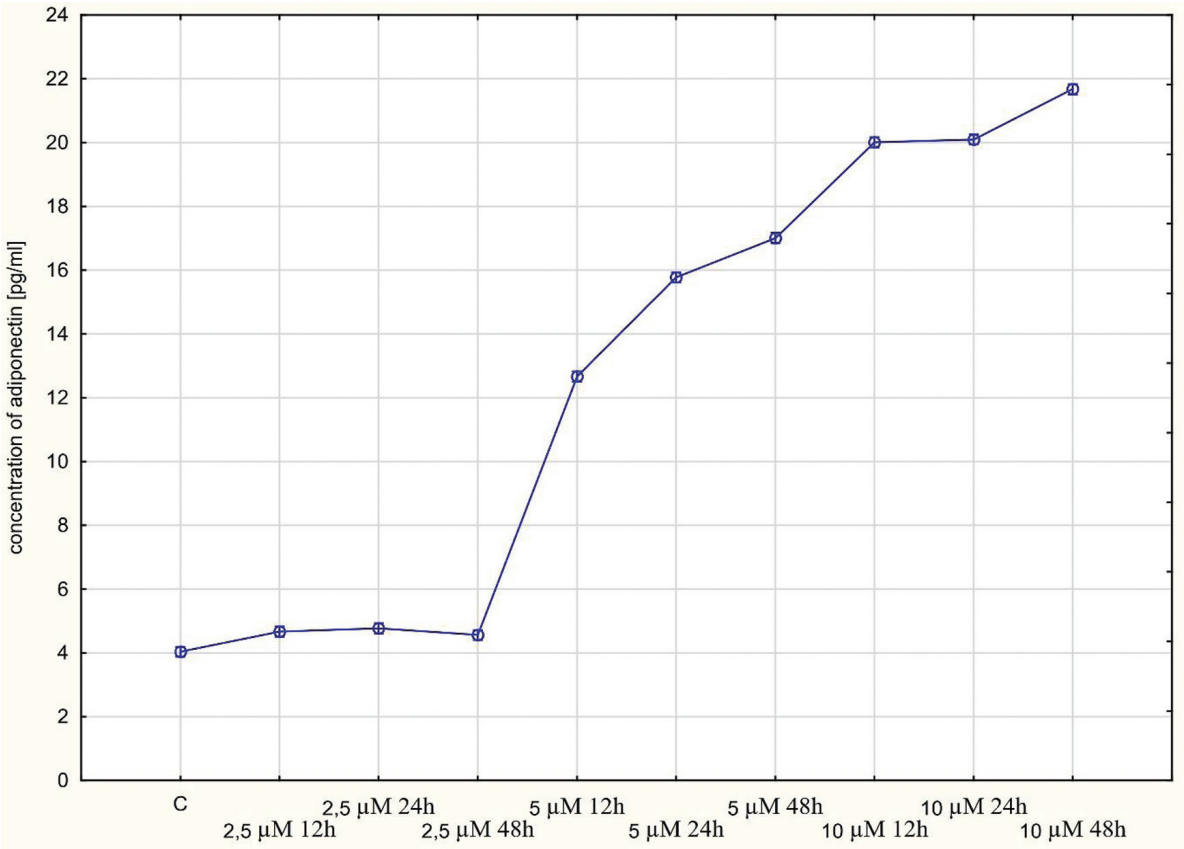

Fig. (2). Differences in the protein level of adiponectin depending on concentration and time of exposition of cisplatin. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

conditioning, the development of effective diagnostic-therapeutic strategies cannot be limited to the analysis of the changes in expression of these genes and the signaling pathways activated by them, which make up, among others, the gripping point for anti-cancer drugs.

Therefore, as part of this work, the expression profile of adiponectin was assessed, the protein whose secretion happens mainly in adipocytes, conditioned by remaining insulin-sensitive [3, 4] in endometrial cancer cells of cell line Ishikawa. Other than this, we analyzed if and how cisplatin, one of the chemotherapy drugs used in advanced forms of endometrial cancer [18, 19] affects the expression profile of adiponectin.

Firstly, based on the results obtained by us, it can be observed that endometrial cancer cells are capable of adiponectin expression, not only on the mRNA level but also on the protein level. Hyun-Seuk *et al.* based on the analysis carried out by themselves on a model of endometrial cancer, confirm the key role of adiponectin in the induction of the neoplastic transformation process [21]. They indicated, that adiponectin is an inhibitor of the proliferation of changed neoplastic cells, as well as increasing the concentration of LKB1 particles [21], which is necessary for activating the AMPK pathway [7-11], through this also affecting the proliferation, adhesion, and migration of neoplastic cells [21]. Observations made by Cai *et al.* confirm that adiponectin is a mediator between the signaling cascades of AMPK/mTOR/S6K1 and stops the proliferation and migration of endometrial cancer cells [22]. For this reason, in the second part of the experiment carried out by us, we decided to analyze whether cisplatin has an effect on the concentration profile of adiponectin in an endometrial cancer cell culture. We also aimed to see if the effect of cisplatin on the level of adiponectin is dependant on the dosage of the drug used and/or the incubation time with the drug. Under the influence of the drug added to the culture, the level of adiponectin not only on the transcriptome level but also on the proteome level increased. However, as far as the increase in the dose of cisplatin is concerned (2.5 μM; 5 μM; 10 μM), the effect on the concentration of adiponectin was more noticeable. The observed tendency for an increase suggests that systemic therapy with the use of cisplatin has an effect on the expression pattern of adiponectin. Therefore, it is possible, that the expected regression in symptoms would be a result not only of the direct destructive actions of cisplatin on DNA or mitochondria [18, 19], but also stopping the proliferation of neoplastic cells, would be a result of an increased level of adiponectin [7, 8, 23]. It is worth noting that no matter the concentration of cisplatin and time of incubation for the cells with the drug, no possibility for a loss of an adequate response to treatment was observed, expressed by a drop in the level of adiponectin compared to its level with a shorter exposition time. This could indicate a relatively low risk of drug resistance connected with cisplatin therapy because of endometrial cancer. Nonetheless, however, it is worth taking into account that the observations made in this study were done on an *in vitro* model. Chitcholtan *et al.* indicated that in the induction of drug resistance on anticancer drugs a key role is played by the microenvironment of the tumor, the different types of cells made by it, which are capable of secreting different types of biologically active compounds such as cytokines, growth factors, affecting the metabolism of neoplastic cells. They also pay attention to, that differences in conclusions about the possibility of a loss of answer to treatment appearing such as cisplatin in the case of *in vitro* research are dependant on the type of endometrial cancer cell line used. Simultaneously, however, they indicate the legitimacy and use of the used cell line for research about the therapeutic potential of anticancer drugs. Unfortunately, in their research, they did not carry out analysis on cell line Ishikawa, which was used in our research [24]. Wang *et al.* analyzed the concentration of adiponectin, with the use of the ELISA technique, on a group of 53 female patients with a diagnosed endometrial cancer and compared it to a group of healthy women, which made up the control of the experiment. They observed a statistically significant ($p<0.001$) decrease in the level in the group with endometrial cancer (2.09 ± 1.24 μg/ml) in comparison to the control (7.59 ± 2.29 μg/ml) [25], indicating the importance of the concentration of adiponectin simultaneously, as an independent molecular marker of the risk of developing endometrial cancer [25, 26]. In turn, Ciortea *et al.* judged the concentration of adiponectin in a coculture arrangement of cell line Ishikawa with Adipose-derived Stem Cells (ASCs) as well as in the culture of only Ishikawa cells. Additionally, they determined the silencing of expression of adiponectin measured with the ELISA technique and compared to the control culture. In turn, in the endometrial cancer cell Ishikawa culture, they noted that in the lysate of the cell, the concentration of adiponectin on the level of around 4.5 ng/ml, and in the post-culture medium the level was around 3 ng/ml [27]. The resulting concentrations of adiponectin obtained by them are concurrent with our observations in the unexposed to cisplatin culture. Analysis of the concentration of adiponectin not only in the endometrial neoplasm but also during anti-neoplastic therapy is important because of the documented anti-angiogenic properties of adiponectin *in vitro* and *in vivo* [28, 29]. This could, therefore, be one of the main probable explanations for the silencing of the expression of adiponectin in endometrial cancer in comparison to the control, as through the development of new blood vessels to neoplastic cells nutrients and oxygen are delivered [30]. In connection with the above, considering the results of changes in the concentration profile of adiponectin observed by us with the observations of other researchers [27-30], a reasonable conclusion is that cisplatin is characterized by the anti-angiogenic mechanism of action. On the basis of obtained results in the context of this study, it can be determined, that the optimal effect of cisplatin is seen with concentrations no lower than 5 μM. Then, statistically significant differences in the concentration of adiponectin can be observed for the first time when compared to untreated cells (control) [31]. Busch *et al.* in their research they used a concentration of 1 μg/ml of cisplatin, which is equal to 3,3 μM of cisplatin, and also Skolekova *et al.* used cisplatin of concentrations 0.5-8 μg/ml, wherein for further stages of the study on the breast cancer cell line they used a concentration of 1 μg/ml [32]. On the basis of this information, it can be inferred, that an effective pharmacotherapy of endometrial cancer can be carried out with the above doses of cisplatin. Other than that, the recorded expression profile of adiponectin when stimulating cells with

cisplatin at a concentration of 10 μM, suggests a stabilization in the working of cisplatin, as between the incubation times of 12 and 24 hours, differences on the protein level in adiponectin were not statistically significant. Next, an increase in its expression of about 1.5 ng/ml can be observed. Furthermore, it can be noticed that the analysis confirms how dynamic the changes are on the molecular level. It also seems that the transcriptome is a substance that reacts to changes quicker than the proteome, in which the changes in expression are more noticeable. Nonetheless, the analysis of the level of adiponectin on the mRNA level and the protein allows us to observe changes in the expression profile in two key points of the genetic information flow. Moreover, the methods of RtqPCR and ELISA used in our work to detect changes in the expression of the assessed factor confirmed the ability to assess the concentration of adiponectin with the use of fundamental, increasingly more available methods of molecular biology. In a substantial way, this increases the chances of using adiponectin in routine diagnosis, not only because it yields reliable results, which was confirmed by comparing our observations with observations of other research teams.

CONCLUSION

Cisplatin, in a significant way, changes the expression profile of adiponectin. Molecular analysis showed, that in the case of endometrial cancer therapy, one should start at a concentration no lower than 5 μM of the drug, it seems, that the appearance of drug resistance to cisplatin in the case of endometrial cancer has a marginal meaning.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Schuster, S.; Cabrera, D.; Arrese, M.; Feldstein, A.E. Triggering and resolution of inflammation in NASH. *Nat. Rev. Gastroenterol. Hepatol.,* **2018**, *15*(6), 349-364. http://dx.doi.org/10.1038/s41575-018-0009-6 PMID: 29740166

[2] Tsochatzis, E.; Papatheodoridis, G.V.; Archimandritis, A.J. The evolving role of leptin and adiponectin in chronic liver diseases. *Am. J. Gastroenterol.,* **2006**, *101*(11), 2629-2640. http://dx.doi.org/10.1111/j.1572-0241.2006.00848.x PMID: 16952281

[3] Ezzidi, I.; Mtiraoui, N.; Mohmmed Ali, M.E.; Al Masoudi, A.; Abu Duhier, F. Adiponectin (*ADIPOQ*) gene variants and haplotypes in Saudi Arabian women with Polycystic Ovary Syndrome (PCOS): A case-control study. *Gynecol. Endocrinol.,* **2020**, *36*(1), 66-71. http://dx.doi.org/10.1080/09513590.2019.1632830 PMID: 31242787

[4] Yanai, H.; Yoshida, H.; Iseli, T.J. Beneficial effects of adiponectin on glucose and lipid metabolism and atherosclerotic progression; Mechanisms and perspectives. *Int. J. Mol. Sci.,* **2019**, *20*(5), 1190. http://dx.doi.org/10.3390/ijms20051190 PMID: 30857216

[5] Dong, Z.; Su, L.; Esmaili, S.; Iseli, T.J.; Ramezani-Moghadam, M.; Hu, L.; Xu, A.; George, J.; Wang, J. Adiponectin attenuates liver fibrosis by inducing nitric oxide production of hepatic stellate cells. *J. Mol. Med. (Berl.),* **2015**, *93*(12), 1327-1339. http://dx.doi.org/10.1007/s00109-015-1313-z PMID: 26153548

[6] Nepal, S.; Park, P.H. Modulation of cell death and survival by adipokines in the liver. *Biol. Pharm. Bull.,* **2015**, *38*(7), 961-965. http://dx.doi.org/10.1248/bpb.b15-00188 PMID: 26133703

[7] Ruan, H.; Dong, L.Q. Adiponectin signaling and function in insulin target tissues. *J. Mol. Cell Biol.,* **2016**, *8*(2), 101-109. http://dx.doi.org/10.1093/jmcb/mjw014 PMID: 26993044

[8] Pagano, C.; Soardo, G.; Esposito, W.; Fallo, F.; Basan, L.; Donnini, D.; Federspil, G.; Sechi, L.A.; Vettor, R. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. *Eur. J. Endocrinol.,* **2005**, *152*(1), 113-118. http://dx.doi.org/10.1530/eje.1.01821 PMID: 15762194

[9] Musso, G.; Gambino, R.; De Michieli, F.; Premoli, A.; Biroli, G.; Fagà, E.; Durazzo, M. The postprandial phase as a link between systemic lipid peroxidation and liver injury in NASH. *Am. J. Gastroenterol.,* **2006**, *101*, 1-9. http://dx.doi.org/10.1111/j.1572-0241.2006.00794.x

[10] Kobayashi, H.; Ouchi, N.; Kihara, S.; Walsh, K.; Kumada, M.; Abe, Y.; Funahashi, T.; Matsuzawa, Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ. Res.,* **2004**, *94*(4), e27-e31. http://dx.doi.org/10.1161/01.RES.0000119921.86460.37 PMID: 14752031

[11] Xu, A.; Wang, Y.; Keshaw, H.; Xu, L.Y.; Lam, K.S.; Cooper, G.J. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J. Clin. Invest.,* **2003**, *112*(1), 91-100. http://dx.doi.org/10.1172/JCI200317797 PMID: 12840063

[12] Nishida, M.; Kasahara, K.; Oki, A.; Satoh, T.; Arai, Y.; Kubo, T. Establishment of eighteen clones of Ishikawa cells. *Hum. Cell,* **1996**, *9*(2), 109-116. PMID: 9183638

[13] Straub, L.G.; Scherer, P.E. Metabolic messengers; adiponectin. *Nat. Metabolism,* **2019**, *1*(3), 334-339. http://dx.doi.org/10.1038/s42255-019-0041-z

[14] Di Zazzo, E.; Polito, R.; Bartollino, S. Nigro; E.; Porcile, C.; Bianco, A.; Moncharmont, B. adiponectin as link factor between adipose tissue and cancer. *Int. J. Mol. Sci.,* **2019**, *20*(4), 839. http://dx.doi.org/10.3390/ijms20040839

[15] Rinaldi, G.; Rossi, M.; Fendt, S.M. Metabolic interactions in cancer: cellular metabolism at the interface between the microenvironment, the cancer cell phenotype and the epigenetic landscape. *Wiley Interdiscip. Rev. Syst. Biol. Med.,* **2018**, *10*(1), e1397. http://dx.doi.org/10.1002/wsbm.1397 PMID: 28857478

[16] Turgeon, M.O.; Perry, N.J.S.; Poulogiannis, G. DNA damage; repair; and cancer metabolism. *Front. Oncol.,* **2018**, *8*, 15. http://dx.doi.org/10.3389/fonc.2018.00015 PMID: 29459886

[17] Kölbl, A.C.; Birk, A.E.; Kuhn, C.; Jeschke, U.; Andergassen, U. Influence of VEGFR and LHCGR on endometrial adenocarcinoma. *Oncol. Lett.,* **2016**, *12*(3), 2092-2098. http://dx.doi.org/10.3892/ol.2016.4906 PMID: 27625708

[18] Randall, M.E.; Filiaci, V.L.; Muss, H.; Spirtos, N.M.; Mannel, R.S.; Fowler, J.; Thigpen, J.T.; Benda, J.A. Gynecologic Oncology Group Study. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: A Gynecologic Oncology Group Study. *J. Clin. Oncol.,* **2006**, *24*(1), 36-44. http://dx.doi.org/10.1200/JCO.2004.00.7617 PMID: 16330675

[19] Manohar, S.; Leung, N. Cisplatin nephrotoxicity: A review of the literature. *J. Nephrol.,* **2018**, *31*(1), 15-25. http://dx.doi.org/10.1007/s40620-017-0392-z PMID: 28382507

[20] Brinton, L.A.; Berman, M.L.; Mortel, R.; Twiggs, L.B.; Barrett, R.J.; Wilbanks, G.D.; Lannom, L.; Hoover, R.N. Reproductive, menstrual, and medical risk factors for endometrial cancer: Results from a case-control study. *Am. J. Obstet. Gynecol.,* **1992**, *167*(5), 1317-1325. http://dx.doi.org/10.1016/S0002-9378(11)91709-8 PMID: 1442985

[21] Moon, H.S.; Chamberland, J.P.; Aronis, K.; Tseleni-Balafouta, S.; Mantzoros, C.S. Direct role of adiponectin and adiponectin receptors in endometrial cancer: *In vitro* and *ex vivo* studies in humans. *Mol. Cancer Ther.,* **2011**, *10*(12), 2234-2243. http://dx.doi.org/10.1158/1535-7163.MCT-11-0545 PMID: 21980131

[22] Cai, Z.F.; Deng, L.; Wang, M.M.; Zhang, J.Q.; Li, L. Effect of AMPK/mTOR/S6K1 pathways and the insulin-sensitizing effect for adiponectin in endometrial cancer cells. *Zhonghua Fu Chan Ke Za Zhi,* **2018**, *53*(8), 554-560. PMID: 30138966

[23] Körner, A.; Pazaitou-Panayiotou, K.; Kelesidis, T.; Kelesidis, I.; Williams, C.J.; Kaprara, A.; Bullen, J.; Neuwirth, A.; Tseleni, S.; Mitsiades, N.; Kiess, W.; Mantzoros, C.S. Total and high-molecular-weight adiponectin in breast cancer: *In vitro* and *in vivo* studies. *J. Clin. Endocrinol. Metab.,* **2007**, *92*(3), 1041-1048. http://dx.doi.org/10.1210/jc.2006-1858 PMID: 17192291

[24] Chitcholtan, K.; Sykes, P.H.; Evans, J.J. The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. *J. Transl. Med.,* **2012**, *10*(1), 38. http://dx.doi.org/10.1186/1479-5876-10-38 PMID: 22394685

[25] Wang, Z.; Gao, S.; Sun, C.; Li, J.; Gao, W.; Yu, L. Clinical significance of serum adiponectin and visfatin levels in endometrial cancer. *Int. J. Gynaecol. Obstet.,* **2019**, *145*(1), 34-39. http://dx.doi.org/10.1002/ijgo.12772 PMID: 30702161

[26] Gong, T.T.; Wu, Q.J.; Wang, Y.L.; Ma, X.X. Circulating adiponectin, leptin and adiponectin-leptin ratio and endometrial cancer risk: Evidence from a meta-analysis of epidemiologic studies. *Int. J. Cancer,* **2015**, *137*(8), 1967-1978. http://dx.doi.org/10.1002/ijc.29561 PMID: 25899043

[27] Ciortea, R.Ă.Z. V A.N., Şuşman; S.; Măluţan, A.M.; Berceanu, C.; Mocan-Hognogi, R. F.; Bucuri, C.E.; Mihu, D. Mesenchymal stem cells derived from adipose tissue and Ishikawa cells co-culture highlight the role of adiponectin in endometrial cancer pathogenesis. Romanian journal of morphology and embryology. *Rev. Roum. Morphol. Embryol.,* **2018**, *59*(4), 1165-1172.

[28] Bråkenhielm, E.; Veitonmäki, N.; Cao, R.; Kihara, S.; Matsuzawa, Y.; Zhivotovsky, B.; Funahashi, T.; Cao, Y. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(8), 2476-2481. http://dx.doi.org/10.1073/pnas.0308671100 PMID: 14983034

[29] Maleki, M.; Ghanbarvand, F.; Reza Behvarz, M.; Ejtemaei, M.; Ghadirkhomi, E. Comparison of mesenchymal stem cell markers in multiple human adult stem cells. *Int. J. Stem Cells,* **2014**, *7*(2), 118-126. http://dx.doi.org/10.15283/ijsc.2014.7.2.118 PMID: 25473449

[30] Alhazzani, K.; Alaseem, A.; Algahtani, M.; Dhandayuthapani, S.; Venkatesan, T.; Rathinavelu, A. angiogenesis in cancer treatment; 60 years' swing between promising trials and disappointing tribulations. *Anti-Angiogen. Drug Discov. Developm.,* **2019**, *4*(4), 34. http://dx.doi.org/10.2174/9781681083971119040005

[31] Busch, C.J.; Becker, B.; Kriegs, M.; Gatzemeier, F.; Krüger, K.; Möckelmann, N.; Fritz, G.; Petersen, C.; Knecht, R.; Rothkamm, K.; Rieckmann, T. Similar cisplatin sensitivity of HPV-positive and -negative HNSCC cell lines. *Oncotarget,* **2016**, *7*(24), 35832-35842. http://dx.doi.org/10.18632/oncotarget.9028 PMID: 27127883

[32] Skolekova, S.; Matuskova, M.; Bohac, M.; Toro, L.; Durinikova, E.; Tyciakova, S.; Demkova, L.; Gursky, J.; Kucerova, L. Cisplatin-induced mesenchymal stromal cells-mediated mechanism contributing to decreased antitumor effect in breast cancer cells. *Cell Commun. Signal.,* **2016**, *14*(1), 4. http://dx.doi.org/10.1186/s12964-016-0127-0 PMID: 26759169