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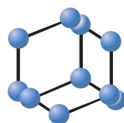
Current Pharmaceutical Biotechnology, 2020, 21, 1119-1128

RESEARCH ARTICLE



Differences in the Expression Pattern of mRNA Protein SEMA3F in Endometrial Cancer *in vitro* under Cisplatin Treatment

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ARTICLE HISTORY

Received: November 29, 2019
Revised: February 04, 2020
Accepted: March 30, 2020

DOI:
10.2174/1389201021666200416102540

Abstract: ***Background***: Semaphorin 3F (SEMA3F) plays a substantial role in carcinogenesis, because of its role in inducing angiogenesis, and creating a microenvironment for the developing tumor.

Objective: The purpose of this work was to assess the impact of cisplatin, depending on the concentration and exposure time on the expression pattern of SEMA3F in an endometrial cancer cell line.

***Materials and Methods*:** Cultures of the Ishikawa endometrial cancer cells were incubated with cisplatin with the following concentrations: 2.5μM; 5μM; and 10μM and for the following periods of time: 12; 24; and 48 hours. Cells not incubated with the drug constituted the control in the experiment. To determine the effect of cisplatin on the expression of SEMA3F, the real-time quantitative reverse transcription reaction (RtqPCR; mRNA) was used, as well as the ELISA assay (protein). The statistical analysis was done with the admission of $p<0.05$.

Results: The silencing of SEMA3F expression on the transcriptome and proteome levels in a culture unexposed to the effects of cisplatin in comparison to endometrial cancer cells under the influence of cisplatin ($p<0.05$) were noted. Along with an increase in the concentration of the drug used, the number of copies of the gene transcript, during the shortest incubation period had a gradual increase. Only for the highest concentration of the drug, substantial statistical differences in the expression of the SEMA3F protein between 24 and 48 hour incubation periods ($p<0.05$) were determined.

Conclusion: Using cisplatin in an endometrial cancer cell culture results in an increased expression of SEMA3F, which advantageously affects the normalization of the neoplastic angiogenic process and lowers the proliferation of the cells making up the mass of the tumor.

Keywords: SEMA3F, endometrial cancer cell line, cisplatin, expression, supplementary molecular marker, gene transcript.

1. INTRODUCTION

Semaphores (SEMA) are a family of proteins, which can be divided into seven subclasses found in vertebrates. Semaphores of classes 1,4,6 and 7 are transmembrane proteins, members of classes 2 and 3 are characterized as secretory proteins, during which members of class 7 are proteins anchored in glycophosphatidylinositol [1]. Semaphores show an ability to interact with neuropilin receptors or plexins, the result of which is the initiation of the mentioned signaling cascades [2, 3]. They have a significant role not only in processes but also mainly in organogenesis, modulating the immunological response, maintaining the rate at which new blood vessels are created, and conditioning the adhesion of cells to the foundation [1-3].

The incorrect expression of certain members of the semaphorin family was determined during the neoplastic transformation process and the process of creating new blood vessels (angiogenesis) and lymphatic vessels (lymphangiogenesis), which are directly tied but does not allow itself to be controlled [4, 5]. One of the members of the aforementioned family of proteins is SEMA3F, the silencing of its' expression is observed in tumors, which constitutes a disadvantageous prognostic marker. It is pointed to that during the neoplastic transformation process, it leads to a deficiency of the aforementioned gene, which is located on chromosome 3p [6]. Therefore, also halting the secretion of SEMA3F results in excessive proliferation of cells in comparison to

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1873-4316/20 $65.00+.00 © 2020 Bentham Science Publishers

Current Pharmaceutical Biotechnology

apoptosis, increasing the potential for metastasis by the modified tumor cell [7, 8].

In physiological conditions, SEMA3F is a strong angiogenic factor, and its effect is caused by competition with the Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-Beta 1 (TGFβ1) for connecting with the neuropilin receptors and continued transduction of signal [9, 10]. The second mechanism through which SEMA3F halts the creation of new blood vessels is based upon promoting the apoptosis of endothelial cells [11, 12].

Analysis of the expression profile of SEMA3F not only in carcinogenesis but also under the influence of the used treatment has a key meaning for a better understanding of the molecular foundation of neoplastic changes and monitoring the effectiveness of therapy [13, 14].

One of the most common gynecological tumours diagnosed is endometrial cancer [15], which in advanced forms is treated with cisplatin [16], the mechanism of which is connected with the induction of damaged DNA, the endoplasmic reticulum as well as mitochondria. In the molecular aspect, the activity of cisplatin strongly influences the pathways connected with apoptosis and oxidative stress [17].

One of the useful models of *in vitro* endometrial cancer used to evaluate changes on the molecular level is the Ishikawa cell line, it was obtained from a well-varied human endometrial adenocarcinoma

The purpose of this work was to assess the impact of cisplatin depending on concentration and exposure time on the expression pattern of SEMA3F in Ishikawa endometrial cancer cells. Moreover, this evaluation may be helpful to indicate the usefulness of SEMA3F as a supplementary molecular marker in endometrial cancer diagnosis and therapies.

2. MATERIALS AND METHODS

2.1. Cell Culture

In this study, the Ishikawa endometrial cancer cell line was used as a material. These cells were exposed to cisplatin at concentrations of 2.5 μM, 5 μM and 10 μM for 12, 24 and 48 hour periods. The untreated cells constituted the control in this work. The cells were routinely grown in the Minimum Essential *Medium* (MEM) supplemented with 2 mM glutamine, 1% Non-Essential Amino Acids (NEAA), and 5% Fetal Bovine Serum (FBS) according to the manufacturer's protocol. Cultures were incubated under conditions of a constant temperature of 37°C and a 5% CO_2 enriched atmosphere. Reagents used in this part of this study were bought from Sigma Aldrich, St Louis, MO, USA. Three replicates of each time and concentration were performed. After 24 hours, cisplatin was added to the culture of cells in six-well plates. The control culture was carried out separately for each time, i.e., for 12, 24 and 48 hours in three replicates.

2.2. XTT Assay

In the first step of this study, proliferation and cell viability of the Ishikawa endometrial cancer cell line under different concentrations of cisplatin and exposure time of the cells to the drug by XTT assay was evaluated (*In Vitro* Toxicology Assay Kit, XTT based; Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer`s protocol.

2.3. Invasion Assay

Invasion of Ishikawa endometrial cancer cells under cisplatin treatment was evaluated by using a 24-well Transwell chemotaxis chamber (Costar, USA) with an 8mm pore size membrane coated with BD Matrigel™Matrix (BDBiosciences, USA). First, the control cells (cells unexposed to cisplatin) and the Ishikawa cultures treated with 2.5 μM, 5 μM, and 10 μM of cisplatin for 12, 24 and 48 hours were suspended in 200 ml serum-free DMEM medium, then these cells were filled in the upper chamber. Next, 600 ml of complete medium was added and incubated for 12 hours. Hematoxylin was used to stain cells and they were microscopically counted.

2.4. Apoptosis Analysis

The Annexin V-FITC/PI apoptosis detection kit (Sigma Aldrich, Product no. APOAF-20TST) was used for analyzing the influence of cisplatin on cell apoptosis. The Annexin V-FITC kit allows for the fluorescent detection of annexin V that is bound to apoptotic cells and additionally quantitative determination through flow cytometry. The AnnexinV-FITC kit uses annexin V conjugated with Fluorescein Isothiocyanate (FITC) in order to label phosphatidylserine sites on the membrane surface. This kit includes Propidium Iodide (PI) for the labeling of cellular DNA in necrotic cells when their cell membrane has been completely compromised. This combination allows for differentiation between early apoptotic cells (annexin V positive, PI negative), necrotic cells (annexin V positive, PI positive), and viable cells (annexin V negative, PI negative).

2.5. Expression of SEMA3F and of TGFβ1 and VEGF (as SEMA3F- Related Genes) on mRNA and the Protein Level

To determine the changes in the expression of *SEMA3F*, and related genes, such as *TGFβ1* and *VEGF* under cisplatin treatment, the real-time quantitative reverse transcription reaction (RTqPCR) and the ELISA assay, were performed.

In the RTqPCR assay, SensiFAST SYBR No-ROX One-Step Kit (Bioline, London, UK) was used, as recommended by the manufacturer. The thermal profile of this reaction was as follows: 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), elongation (72ºC for 5 s). As an endogenous control, β- actin (*ACTB)* was used.

The second stage of this experiment was associated with indicating the influence of cisplatin on the expression profile of SEMA3F on the protein level through the ELISA assay.

The SEMA3F (Cat.No MBS161003, MyBioSource, San Diego, CA 92195-3308, USA) kit was used. The assay was performed at room temperature after all necessary reagents, standard solutions and previously used samples were prepared. Standards containing the biotinylated antibody were added to each well at 50μl. In turn, 10 ml of the anti-

SEMA3F antibody was added to the wells with the introduced samples in the amount of 40 µl, followed by 50 µl of streptavidin-HRP solution. The entirety was incubated for 60 minutes at 37°C, after which the wells were washed 5 times with a wash buffer (60 seconds/wash). In the next step, 50 µl of substrate A solution per well was added, followed by the addition of 50 µl of substrate solution B per well and again incubated for 10 minutes at 37°C in the dark. After this time, 50 µl of stopping solution was added, which resulted in a change in the color of the well contents from blue to yellow. In the last step, the optical density was determined for each sample at 450 nm and the concentration of SEMA3F was determined depending on the dose of cisplatin and the time of incubation of the cells with the drug.

2.6. Analysis if Cisplatin Exerts its effect *via* SEMA3F Signaling Pathways

The last step of our study was associated with assessing if cisplatin exerts its effect *via* SEMA3F signaling pathways by using target-specific 19-23 nucleotide siRNA oligo duplexes designed to knock down *SEMA3F* expression (SEMA3F siRNA Human; Catalog # MBS8213103, MyBioSource, San Diego, CA, USA) and 21 nucleotide siRNA Negative Control (Catalog# MBS8241404, MyBioSource, San Diego, CA, USA). Both untreated and exposed to cisplatin endometrial cancer cells were transfected by SEMA3F siRNA according to instruction. In order to confirm the influence of cisplatin on SEMA3F- related paths, the expression of SEMA3F *via* RTqPCR and the ELISA assay were assessed.

2.7. Statistical Analysis

The licensed version of Statistica 13.0 PL (StatSoft, Cracow, Poland) was used in the statistical analysis of data related to changes in the expression profile of SEMA3F.

As the Shapiro-Wilk test ($p<0.05$) confirmed the normality of the data distribution, the next steps of analysis were made by using parametric tests. The ANOVA variance test was also conducted, and the analysis indicated that the differences in the results were statistically significant and the posthoc Tukey test was also performed ($p<0.05$).

3. RESULTS

To assess the XTT assay results, the absorption value observed in the control cell (untreated endometrial cancer cells to cisplatin) cultures was assumed to be 100%. It was used as a comparison value for the cells exposed to the drug. The results showed that regardless of the concentration of cisplatin used in the experiment, the number of viable cells decreases as the incubation time increases. It can be observed that cisplatin in the concentration of 5 µM causes the death of about 50% of endometrial cells incubated with it (half maximal inhibitory concentration; IC50). Statistically significant differences were showed between the cells treated with cisplatin in comparison to the control cell culture (Fig. **1**; $p<0.05$).

Results of the invasion assay showed that cisplatin in the range of concentrations used statistically significantly inhibited Ishikawa cell invasion when compared to a control culture ($p<0.05$; Fig. **2**). The fastest progressive decline in invasive cells was observed in the culture exposed to 5 µM cisplatin (from 61.9% after 12 hrs by 42.6% after 24 hrs to 41.02% of invasive cells after 48 hour-long incubation time with the drug). The obtained results also showed that the percentage of non-invasive cells was increasing with drug concentration and incubation time with it (Fig. **2**).

The next stage of our work was to evaluate endometrial cancer cell apoptosis induced by cisplatin. As shown in Fig. (**3**), cisplatin increased the percentage of apoptotic cells in a dose and in a time-dependent manner in endometrial cancer cells compared to the control culture ($p<0.05$).

In turn, Table **1** presents changes in the expression of the *SEMA3F* mRNA; the gene coded by this protein depending on the dose of cisplatin; time of exposure of the endometrial cancer cell to the effect of the drug.

First of all, it should be noted that not only on the transcriptome level but also on the proteome level, a decrease in

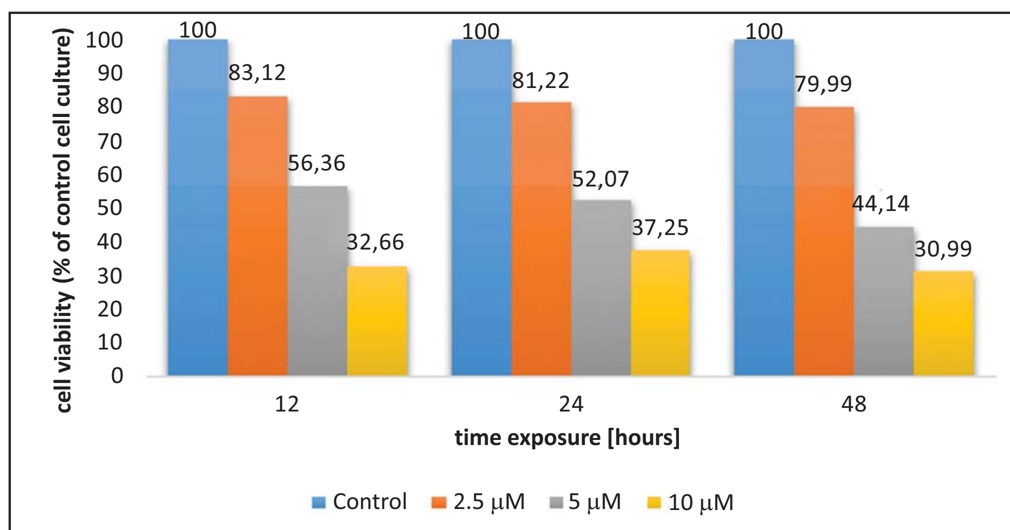

Fig. (1). The results of cytotoxicity and proliferation XTT assay in Ishikawa endometrial cancer cells under cisplatin treatment. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

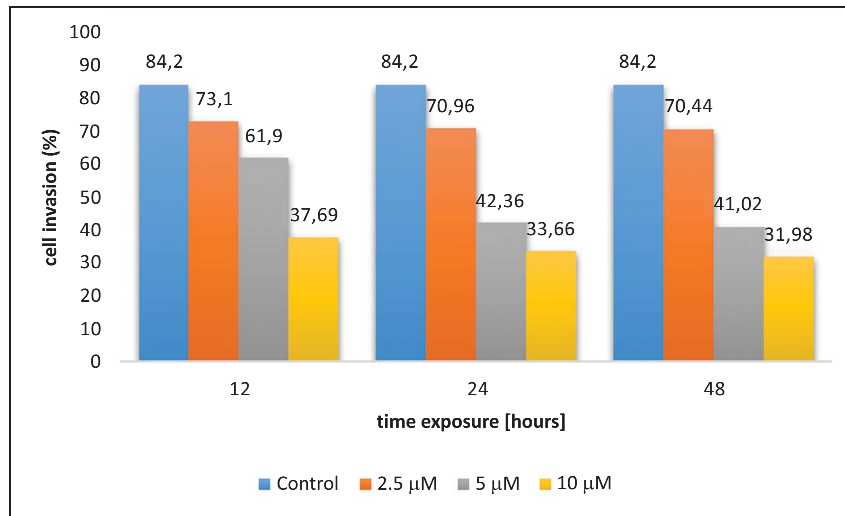

Fig. (2). The effect of cisplatin on Ishikawa endometrial cancer cells invasion. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

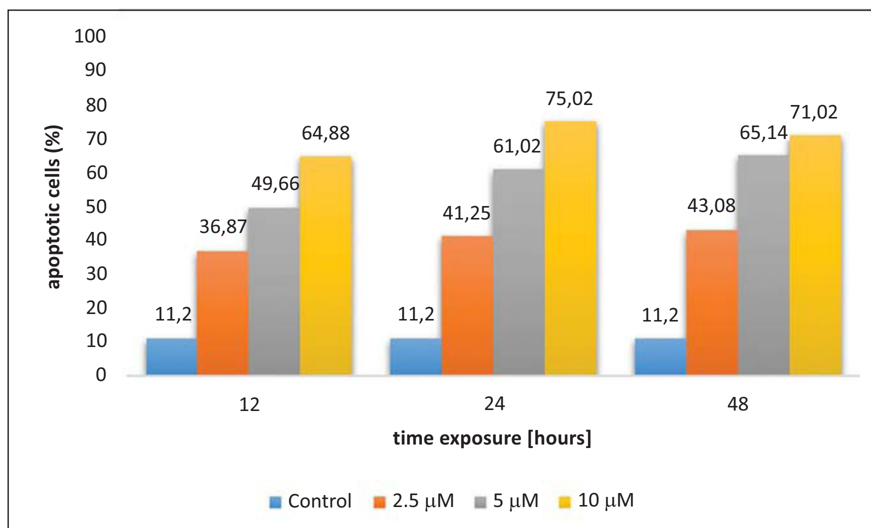

Fig. (3). The proapoptotic influence of cisplatin treatment in Ishikawa cells. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

the expression of SEMA3F in the unexposed to cisplatin culture was observed, when compared to the endometrial cancer cells under the effect of the chemotherapeutic agent ($p<0.05$).

Concerning the matter of the concentrations of cisplatin used, it can be observed that alongside the elongated exposure time of the cells to the drug, the expression on the mRNA level increases successively, similarly to what happens on the protein level. Nonetheless, for the concentration of 5 µM, it can be determined that the exposition lasting an entire day causes a decrease in expression in comparison to the 12-hour incubation, wherein extending the amount of time the drug affects the endometrial cancer cells to 48 hours caused an increase in the transcriptional activity of SEMA3F.

Based on the obtained results, it can also be observed that with an increase in the concentration of the drug used, the number of the transcription gene copies increases, with the shortest amount of incubation time, it is subjected to a gradual increase.

Also, on the protein level, it can be determined that cisplatin induces changes on the SEMA3F level, no matter the length of time or dosage of the drug used ($p<0.05$). Statistical analysis indicates that there were statistically significant changes between the level of SEMA3F between certain incubation times, which are induced at the same concentration of cisplatin. However, only for the highest concentration of the drug, substantial statistical differences in the expression of SEMA3F between the times of 24 and 48 hours of incubation ($p<0.05$) were determined, for the other concentrations in this comparison, this wasn't the case ($p>0.05$).

In turn, the last step of the molecular analysis was to make sure that cisplatin exerts an effect via SEMA3F dependent signal pathways. The results of the expression profile of mRNA *TGFβ1* and *VEGF* under cisplatin treatment were presented in Table **2**.

Table 1. The expression profile of SEMA3F depending on the concentration and the amount of exposure time of endometrial cancer cells when undergoing cisplatin treatment.

Concentration of Cisplatin [µM]	Time [Hours]	RTqPCR (Copy Number of *SEMA3F/*1µg of total RNA		RTqPCR (Copy Number of β-actin/1µg of total RNA		ELISA Assay SEMA3F [ng/ml]	
		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	**12**	2579974	52669	72770	1012	7.991	0.026
0	**24**	2461323	45863	64139	694	7.715	0.058
0	**48**	2349829	71402	15083	745	6.71	0.066
2.5	**12**	3732235[a]	335561	72403	968	9.230[a]	0.06
	24	2703216[a.b]	54062	12655	1002	10.688[a .b]	0.019
	48	5144625[a.c.d]	156403	61875	1058	10.830[a.d]	0.036
5	**12**	9371474[a]	1670092	132600	786	10.960[a]	0.053
	24	6967012[a.b]	174888	150900	30012	11.377[a]	0.020
	48	8740369[a.c.d]	265717	128500	21000	11.487[a.d]	0.021
10	**12**	19974217[a]	399464	77825	985	11.524[a]	0.007
	24	174913193[a.b]	27104177	38083	1047	11.523[a.c]	0.006
	48	19319607[a.c.d]	403948	180306	698	12.103[a.c.d]	0.012

[a] – statistically significant differences in expression of SEMA3F between cells exposed to cisplatin *vs* control p<0.05.
[b] – statistically significant differences in expression of SEMA3F between 12 *vs* 24 hours exposition of cisplatin p<0.05.
[c] – statistically significant differences in expression of SEMA3F between 24 *vs* 48 hours exposition of cisplatin p<0.05.
[d] – statistically significant differences in expression of SEMA3F between 12 *vs* 48 hours exposition of cisplatin p<0.05.

Table 2. The expression profile of *TGFβ1* and *VEGF* depending on the concentration and exposure time of endometrial cancer cells when undergoing cisplatin treatment.

Concentration of Cisplatin [µM]	Time [Hours]	RTqPCR (Copy Number of *TGFβ1/*1µg of Total RNA		RTqPCR (Copy Number of *VEGF/*1µg of Total RNA	
		Mean	Standard Deviation	Mean	Standard Deviation
0	**12**	30890	1269	2146342	36584
0	**24**	30785	5844	21106342	42105
0	**48**	31025	5478	2136342	35896
2.5	**12**	37169[a]	4985	546980[a]	61259
	24	37573[a,b]	3654	444356[a,b]	12586
	48	42153[a,c,d]	3568	426187[a,c,d]	25698
5	**12**	46078[a]	36526	83747[a]	12569
	24	88714[a,b]	27542	79753[a,b]	36058
	48	91817[a,c,d]	25697	59434[a,c,d]	41252
10	**12**	333500[a]	20145	41116[a]	2569
	24	757429[a,b]	14371	42404[a,b]	2485
	48	102415[a,c,d]	125869	40265[a,c,d]	2369

[a] – statistically significant differences in expression between cells exposed to cisplatin *vs.* control p<0.05.
[b] – statistically significant differences in expression between 12 *vs.* 24 hours exposition of cisplatin p<0.05.
[c] – statistically significant differences in expression between 24 *vs.* 48 hours exposition of cisplatin p<0.05.
[d] – statistically significant differences in expression between 12 *vs.* 48 hours exposition of cisplatin p<0.05.

It can be observed that cisplatin changes the expression profile of SEMA3F-related genes on the transcriptome level. The analysis showed that *TGFβ1* was upregulated, while *VEGF* was down regulated in the cell cultures exposed to cisplatin. The effect of the drug on the expression of selected genes was confirmed by more visible changes with a higher dose of cisplatin and a longer exposure time. Statistical analysis showed that differences in the expression pattern of mRNA *TGFβ1* and *VEGF* between Ishikawa endometrial cancer cell line cultures incubated with cisplatin compared to a control culture constituting of untreated cells are statistically significant ($p<0.05$; Table **2**).

Table 3. The expression profile of SEMA3F after transferred to cell culture SEMA3F siRNA.

The Concentration of Cisplatin [μM]	Time [Hours]	RTqPCR (Copy Number of *SEMA3F*/1μg of Total RNA SEMA3F siRNA Absent	RTqPCR SEMA3F siRNA Present	% of Silencing in the Expression of SEMA3F between Absent *vs.* Present of siRNA Cell Culture	ELISA Assay SEMA3F [ng/ml] SEMA3F siRNA Absent	ELISA Assay SEMA3F siRNA Present	% of Silencing in Expression of SEMA3F between Absent *vs.* Present of siRNA Cell Culture
0	12	2579974	618162	76,04	7.991	0.90	88.72
0	24	2461323	615331	75,00	7.715	0.87	88.72
0	48	2349829	563959	76,00	6.71	0.76	88.72
2.5	12	3732235	665084	82,18	9.23	1.04	88.72
	24	2703216	378450	86,00	10.688	1.21	88.72
	48	5144625	703219	86,33	10.83	1.22	88.72
5	12	9371474	1780580	81,00	10.96	1.24	88.72
	24	6967012	1372432	80,30	11.37	1.28	88.72
	48	8740369	1660670	81,00	11.487	1.30	88.72
10	12	19974217	4194586	79,00	11.524	1.30	88.72
	24	174913193	30434896	82,60	11.523	1.30	88.72
	48	19319607	3477529	82,00	12.103	1.37	88.72

The last part of this work confirmed the effect of cisplatin *via* SEMA3F - signaling pathways by evaluating changes in the expression of SEMA3F on mRNA and the protein levels. According to the obtained results, presented in Table **3**, it can be observed that the transfected endometrial cancer cells via SEMA3F siRNA resulted in a decrease in the expression of SEMA3F on the transcriptome and proteome level of about 75-88% of the expression level observed in the cell culture without the SEMA3F siRNA vector (Table **3**).

4. DISCUSSION

The continual development of molecular biological methods and techniques creates the ability to come to know the molecular foundations of illnesses, which is also incredibly vital because of the introduction of new drugs to medicine, these are made from substances which specifically interact with factors that are key to the process and the signaling pathways which are activated by them [17].

SEMA3F has a negative effect on the phenomenon of new blood vessels being created, through which oxygen and other substances are delivered to the tumor. This indicates that SEMA3F also has a regulatory role in the context of lymphomagenesis, as well as a direct effect on the proliferation of changed tumor cells, which should be directed down the path of apoptosis. However, during the process of neoplastic transformation, it is observed that SEMA3F has a lowered activity, through which the tumor gains an opportunity for further development and gaining metastatic potential [4, 11, 12].

Beuten *et al.* observed that, in a group of 789 men with prostate cancer, a higher concentration of SEMA3F and SEMA3B were characteristic of patients in whom the effectiveness of therapy was smaller than the expected value. Similarly, these authors also acknowledged that the expression of SEMA3F and SEMA3B could be markers that suggest a negative prognosis of disease remission duration [18]. In turn, Li *et al.* indicated that in a group of 198 patients, the expression of SEMA3F in the group of patients with a low- and intermediate-risk of localized prostate cancer was not significantly different in the assessed groups and did not correlate with experience. The materials used for this study were biopsies obtained during a radical prostatectomy [19]. An interesting observation regarding the relationship between the expression of SEMA3F and the intensification of cancerous changes as well as regarding the answer to treatment were made by Drenberg *et al.*, together with the intensification of the severity and advancement of changes in the duration of ovarian cancer they determined an ever lower expression of SEMA3F, and in some of the patients a complete silencing of expression. Simultaneously they indicated that only the severity of the tumor correlated with a decreased concentration of SEMA3F and advancement of the disease [20]. However, there is a lack of research regarding the effects of cisplatin on the expression of SEMA3F in the *in vivo* model.

Bearing in mind the cited results of research [18-20] together with the observations cited in our own study, it seems that the level of expression of SEMA3F is dependent not only on the aggressiveness of cancer but also on its location. It is important to keep in mind the observations made by Beuten *et al.*, which contrast with the generally accepted role of SEMA3F [6-12]. This could indicate that SEMA3F could be an advantageous or disadvantageous marker of survival or response to treatment depending on the biological context, as is in the case of, for example, Transforming Growth Factor-Beta 1 (TGF-β1) [21].

Therefore, the carried-out treatment should also have a regulatory effect on genes and proteins with a key role in the neoplastic process and lead to the opening of their expression

on the same or close to the physiological level. Due to this, in the case of this work, we decided to assess the changes in the expression of SEMA3F under the influence of cisplatin, as well as to analyze if the molecular effect, measured by the changes in expression of SEMA3F, of cisplatin is determined by its' dosage or by the amount of time it was exposed to the drug. By using the commercially available endometrial cancer cell line Ishikawa [22], we were able to analyze the direct effect of the drug, isolated from the effects of the outside factors, on the expression profile of SEMA3F, not only on the transcriptome but also proteome level.

The results we gained are concurrent with the observations made by other research teams, as in cells not treated by cisplatin, the expression of SEMA3F was significantly higher than in the culture exposed to the drug [6]. Jiang *et al.* indicate that SEMA3F in physiological conditions functions as a suppressor of the carcinogenic induction process [23]. We observed that using the smallest dosage of cisplatin in this study already caused an increase in the expression of SEMA3F which suggests that there is a therapeutic benefit during treatment even in small doses. The conclusion from this seems evident when you consider the fact that molecular changes appear before phenotypic ones [24]. Similarly, noting the changes on the molecular level after a relatively short time of exposition of the endometrial cancer cells to the drug indicates that the effects on the phenotypic level will be evident in a later period. This also shows that it's valuable to implement a routine diagnosis and therapy of the molecular signs, as, based on them appearing first, it is possible to predict the best treatment available [25]. Therefore, the modern approach to medicine should also be concentrated on finding new supplementary molecular markers, determining their referential range of concentrations and implementation for *in vivo* markers,

In our previous studies, we focused on determining the influence of directed molecular therapy (tumor necrosis factor - α, TNF- α paths and adalimumab) on the expression profile of genes of signal pathways with a key role in psoriasis pathogenesis [24]. We concluded that along with the increase in incubation time of the cells with the drug, its influence, expressed by the number of differentiated ID mRNA in the culture with adalimumab, in comparison with the control, undergoes a gradual increase. However, when speaking about the observations made in this study, we did not conclude that increasing the exposition time for endometrial cancer cells to cisplatin resulted in a change in the expression profile of *SEMA3F* mRNA and SEMA3F protein towards the one noted for the control culture (cells not treated by the drug). This observation is also substantial from the point of view of the aforementioned loss of responsiveness to the treatment during the use of cisplatin. Galluzi *et al.* indicate that the appearance of resistance to cisplatin treatment is characterized by being complex and dependent on many factors, indicating that genetic and epigenetic factors have a key meaning. Similarly, in order to fight against the loss of the therapeutic effect of cisplatin, it would be worth having a multidimensional strategy [26]. One of the potential mechanisms which may affect the lack of any resistance to cisplatin by the cells of cell line Ishikawa being observed could be connected with the lack of induction by the adaptation mechanisms in answer to adding cytotoxic drugs to the culture [27, 28]. This observation makes up an important factor in carrying out a thorough evaluation of the effectiveness of cisplatin throughout female patients with diagnosed endometrial cancer. On the other hand, the observed pattern of expression of SEMA3F also indicates that the therapeutic influence of cisplatin is spread out over time.

Taking into account the research of Ottes Vasconcelos *et al.* carried out on an *in vitro* model of a melanoma cell line resistant to cisplatin, a probable factor allowing endometrial cancer cells used in our study to continue to be affected by cisplatin is continually having a quite high level of docosahexaenoic acid (DHA, 22:6ω-3) and eicosapentaenoic acid (20:5ω-3) [29].

The observed tendency for the expression levels of the gene and protein of SEMA3F continuing to increase highlights the importance of carrying out therapy in a systematic way, and the full therapeutic effect will be achieved at a later time during the therapy. Above all, the noted by us changes in concentration of SEMA3F no matter the dosage of cisplatin highlights the meaningfulness of personalization of treatment and individual choice of dosage for the patient [30, 31]. Kohei *et al.* also brings attention to having an individualist approach to the treatment of patients, including oncological patients. A group of 18 patients with a metastatic urothelial carcinoma who were recommended gemcitabine-cisplatin therapy were classified depending on the criteria of drug dosage and also intervals between individual intervals during therapy. They concluded that it is reasonable to choose an individual dose and treatment scheme for each patient based on how the patient responds to treatment [32]. In turn, Yamada *et al.* based on a carried out randomized experiment of the III stage on a group of patients with advanced gastric cancer, also highlights the effectiveness of cisplatin as a first-choice drug in chemotherapy. They analyzed if adding docetaxel to the treatment plan using cisplatin and S-1 would have a positive influence on the survival period of the patients. In summary, they concluded that adding another chemotherapeutic agent did not have a substantial effect on the survival period of the patient [33].

One of the factors that limit the use of cisplatin in oncology is the high hepatotoxicity of the drug. As a result of this, there is a search for the possibility to lower the unfavorable effect of the chemotherapeutic agent. Mondal *et al.* indicate that the simultaneous application of cisplatin together with ethanolic extract of Boldo (*Peumus boldus*) decreases the hepatotoxic effect of the drug, simultaneously decreasing its anticancer potential. The assessment was performed on three cancer cell lines (A549, HeLa and HepG2) as well as on one correct line (WRL-68) and was exposed to each of the compounds together and separately [34]. Likewise, the observations of Shen *et al.* indicate that supplementing cisplatin treatment through adding to the treatment scheme Yangfei Kongliu Formula (YKF), a compound Chinese herbal medicine, results in increased effectiveness of therapy. These researchers determined a synergistic effect between cisplatin and YKF caused mainly by the pathway dependent on TGF-β1. They observed that each of these drugs has anticancer potential, but at the same time, their application in the case of lung cancer resulted in larger changes in the assessed

genes than in the case of using all of these drugs separately. Shen *et al.* noted, above all, a decrease in the expression of *TGF-β1, Smad3*, interleukin 2 (IL-2) and TNF-α as well as overexpression of Smad7 in comparison to the control [35].

Proof that the detection of changes in the expression profile of SEMA3F may constitute a promising diagnostic-therapeutic marker is also constituted by the observations of Nguyen *et al.* who confirmed, that a lowered expression level of SEMA3F in the cases of endometrial cancer [36] or also the observations of Scheerer *et al.* indicating, that previous inductions of SEMA3F were through inflammation [37]. Above all, in our previous study, we analyzed the differences in the expression pattern of SEMA3F in biopsies obtained from female patients with a diagnosed endometrial cancer at different stages of its histological advancement and compared to a control. We observed that, in neoplastic samples, the level of SEMA3F was substantially statistically lower than in the control [38]. Therefore, the expression pattern of SEMA3F noted by us in this study confirms that a beneficial, expected phenomenon during pharmacotherapy is an increase in the expression of SEMA3F. Based on our observations, it seems that cisplatin therapy could accompany an increase in the concentration of estrogens. It was confirmed that the endometrial cancer cell line Ishikawa shows an ability to express estrogens [39, 40]. Likewise, it indicates that estrogens are an essential way to answer for an increase in SEMA3F expression [41, 42]. Furthermore, another possible reason for determining an increase in the expression of SEMA3F during the use of cisplatin can be induced by hormonal drug changes.

Moreover, our observations confirm that not only transcriptomes but also proteomes are dynamic systems, which react quickly to changes. The performed analysis also confirm the apoptotic effect of cisplatin on endometrial cancer cells and the influence of the drug on SEMA3F- signaling paths.

CONCLUSION

Analysis of the changes in the expression of SEMA3F on the mRNA and protein level in endometrial cancer *in vitro* model indicates that using cisplatin is an effective therapeutic option in this type of neoplasm. Adding it to a culture, no matter the concentration, increased the expression of SEMA3F. Our observations highlight the need for personalizing the approach to therapy and treatment and also form the basis for further experiments.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data will not be shared due to the fact the third-party rights and commercial confidentiality.

FUNDING

None.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

All authors were responsible for the concept and design of the study, collection and collation of data, analysis and interpretation of data, writing of the article, reviewing, and final reviewing of this article and graphics performance.

We would like to thank Mr. Oskar Ogloszka, for improving our work and language correction.

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