Assessment of concentrations of sTRAIL ligand and its receptors sTRAIL-R1 and sTRAIL-R2 – markers monitoring the course of the extrinsic pathway of apoptosis induction: potential application in ovarian cancer diagnostics

Aleksandra Mielczarek-Palacz, Justyna Sikora, Zdzisława Kondera-Anasz

School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Immunology and Serology, Medical University of Silesia, Katowice, Sosnowiec, Poland

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Corresponding author:
Aleksandra Mielczarek-Palacz
School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Immunology and Serology
Medical University of Silesia
8 Jedności St
41-200 Sosnowiec, Poland
Phone: + 48 32 3641240
E-mail: apalacz@sum.edu.pl

Abstract

Introduction: TNF-related apoptosis-inducing ligand (TRAIL) together with its receptors are involved in activation of the extrinsic pathway of apoptosis. Due to the special role of the apoptosis pathway in pathogenesis of ovarian cancers, the aim of the study was to assess concentrations of sTRAIL, sTRAIL-R1 and sTRAIL-R2 in serum of affected women.
Material and methods: The study group included 85 women with diagnosed ovarian tumors: 35 women with ovarian serous cystadenoma, 15 women with ovarian teratoma and 35 women with serous cystadenocarcinoma. The control group consisted of 30 healthy women. Concentrations of studied parameters were measured by ELISA methods.
Results: Serum levels of all studied parameters were higher in serum of women with ovarian tumors than in the controls, but their concentrations varied depending on the clinical diagnosis. The highest concentration of TRAIL was found in serum of women with ovarian cancer, the highest sTRAIL-R1 level in serum of women with ovarian mature teratoma, and the highest sTRAIL-R2 level in serum of women with ovarian serous cystadenoma.
Conclusions: The state of immunosuppression accompanying neoplastic disease depends on the extrinsic pathway of apoptosis induction in the TRAIL/TRAIL-R system. Determination of TRAIL-R1 and TRAIL-R2 levels may prove to be useful in ovarian tumor differential diagnostics, which requires further research.

Key words: sTRAIL, sTRAIL-R1, sTRAIL-R2, ovarian cancer.

Introduction

In the course of ovarian cancers the disorders of immune response directed against neoplastic cells, in particular apoptosis inhibition, play a crucial role. So far many mainly proteins which determine the course and execution of programmed cell death have been described. Members of the tumor necrosis factor (TNF) superfamily of cytokines are non-covalently linked trimers that play important roles in regulating between cell death and survival [1]. From among them, a particular role falls to

proteins involved in the activation of the extrinsic pathway of apoptosis [2, 3]. One of the proteins from this family is TNF-related apoptosis-inducing ligand (TRAIL, APO2-L) together with its receptors. TRAIL is a type II transmembrane protein consisting of 281 amino acids, and its intracellular part contains a 70-amino acid domain called "the death domain" [4]. Membrane-bound TRAIL can be cleaved to a soluble form of the protein species through the action of metalloproteases. Additionally, the soluble TRAIL (sTRAIL) as well as the membrane-bound form possesses biological activity. TRAIL is able to form trimers, like all the other ligands of the TNF family [5].

TRAIL-induced apoptosis activation depends on its binding to a TRAIL-R receptor. There are five types of TRAIL receptors: TRAIL-R1 (DR4), TRAIL-R2 (DR-5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2) and osteoprotegerin (OPG). TRAIL-R1 and TRAIL-R2 contain a cytoplasmic death domain and transduce apoptotic signals, while TRAIL-R3 and TRAIL-R4, as well as OPG, lack the intracellular death domain and apoptosis-inducing capability and have been proposed to function as decoy receptors, protecting normal cells from apoptosis [6]. TRAIL induces the formation of a pro-apoptotic death-inducing signaling complex (DISC) via its death receptors. The formation of the DISC activates caspase-8, which requires further signal amplification through the mitochondrial pathway for efficient activation of effector caspases in malignant cells [7, 8].

Both the pro-apoptotic ligands TRAIL-R1 and TRAIL-R2 may occur also in a soluble form (sTRAIL-R) as a result of exfoliation of this molecule from the cell surface [9, 10].

Previous research draws attention to the possibility of monitoring the mechanisms of apoptosis in the course of neoplasms. Research on the assessment of the diagnostic and therapeutic usefulness of proteins involved in apoptosis is constantly in progress. The regulation of this process may turn out to be possible by the measurement of molecules released to the blood serum. Due to the special role of the extrinsic apoptosis pathway in ovarian cancer pathogenesis, the aim of the study was to assess the concentrations of sTRAIL, sTRAIL-R1 and sTRAIL-R2.

Material and methods

The study group included 85 women, aged 21 to 62 (mean age: 47.30 ±12.60 years) with diagnosed ovarian tumors.

Clinical follow-up information, such as treatment regimen, response to treatment, physical examination results, imaging results, date and type of recurrence, as well as personal and family history, were available through medical chart abstraction and a questionnaire completed at enrolments. The group included 35 women with diagnosed ovarian serous cystadenoma, 15 women with ovarian teratoma and 35 women with serous cystadenocarcinoma at stage Ia as per WHO criteria. All patients were admitted to the Clinic of Gynecology and Obstetrics in Ruda Slaska of the Medical University of Silesia in the period from 2009 to 2012, for diagnostic or therapeutic treatment of ovarian cancer. Staging employed the criteria recommended by the International Federation of Gynecology and Obstetrics (FIGO). The studied group encompassed women in whom clinical diagnosis of ovarian tumor was confirmed by the results of histopathological examination and other coexisting reproductive organ conditions were excluded. The control group consisted of 30 healthy women aged between 24 and 60 (mean age: 45.50 ±8.90 years) in whom no pathological changes within the reproductive system were detected. They are patients of the Specialist Clinic for Women in SPZOZ in Ruda Slaska. Women included in the study had no chronic circulatory, autoimmune or neoplastic disease and had not been taking any anti-inflammatory or immunomodulatory medications in the preceding two months.

In all the women the blood serum was the research material. In the study group blood was taken from women after establishing the clinical diagnosis, before surgery. Blood was taken in the morning from the cubital vein, to a clot tube, in order to obtain the serum. Thirty minutes after taking the blood, it was centrifuged at 1500 × g for 15 min. The serum obtained in this manner was kept in small portions at a temperature of –80°C until the tests. The blood of the control group women was taken when the women came for check-up and the same procedure of biological material collection was applied. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of the studied parameters. The following kits were used for this purpose: TRAIL/Apo2L, TRAIL-R1/DR4 and TRAIL-R2/DR5 ELISA by DIACLONE (Besancon, France). Test sensitivity was respectively 64 pg/ml, 8 pg/ml and 6 pg/ml. All the women who participated in the study consented to conducting the research. The approval of the Ethics Committee of the Medical University of Silesia in Katowice was obtained.

Statistical analysis

The obtained results were subjected to statistical analysis by applying the computer programs Statistica for Windows 10.0 and Microsoft Excel. In order to verify the distribution of the obtained results, the Shapiro-Wilk test was used. After establishing that the obtained results corresponded to the normal distribution, the arithmetic mean ($\bar{x}$) and standard deviation (SD) were calculated

for each parameter. The mean values of the studied parameters in the studied group and the control group were compared by means of Student's *t*-test. Correlations were tested by Spearman's rank correlation test and presented as the correlation coefficient (*r*). The level of $p \leq 0.05$ was considered statistically significant.

Results

sTRAIL

In the serum of all the women both from the study group and the control group sTRAIL was found. All data for studied parameters are presented in Table I. In women with ovarian cancer the average concentration of sTRAIL was 2354.92 ±727.29 pg/ml and it was significantly higher than in the control group, in which the average value was 696.67 ±173.67 pg/ml ($p < 0.0001$). The concentration of the studied parameter varied depending on the clinical diagnosis of neoplasm. The highest average concentration was found in the serum of women with ovarian cancer (3136.53 ±289.52 pg/ml), and it was significantly higher than the average concentration in the group of women with ovarian serous cystadenoma (1711.74 ±306.09 pg/ml) ($p < 0.0001$) and ovarian mature teratoma (2031.93 ±263.38 pg/ml) ($p < 0.0001$).

sTRAIL-R1

In the control group the concentration of sTRAIL-R1 was below test sensitivity. In women with ovarian cancer the average concentration of sTRAIL-R1 was 25.35 ±9.16 pg/ml and it was significantly higher than in the control group ($p < 0.0001$). The highest average concentration was found in the serum of women with ovarian mature teratoma (31.09 ±9.96 pg/ml), and it was significantly higher than the average concentration in the group of women with ovarian serous carcinoma (26.65 ±8.77 pg/ml) ($p < 0.01$) and ovarian serous cystadenoma (21.59 ±7.66 pg/ml) ($p < 0.01$).

sTRAIL-R2

In the control group the concentration of sTRAIL-R2 was below test sensitivity. In women with ovarian cancer the average concentration of sTRAIL-R2 was 69.69 ±6.00 pg/ml and it was significantly higher than in the control group ($p < 0.0001$). The highest average concentration was found in the serum of women with ovarian serous cystadenoma (72.17 ±7.20 pg/ml), and it was significantly higher than the average concentration in the group of women with ovarian serous carcinoma (67.87 ±4.42 pg/ml) ($p < 0.001$) and ovarian mature teratoma (68.16 ±4.10 pg/ml) ($p < 0.01$).

We then calculated the concentration ratio of sTRAIL-R1 and sTRAIL-R2, taking into consideration that both receptors were involved in signal transduction into the cell. The lowest value of ratio was in serum of women with ovarian serous cystadenoma (0.31). In women with ovarian mature teratoma the TRAIL-R1/R2 ratio was at the same level (0.45). The highest ratio value was in serum of control women (1.0). The sensitivity and specificity of each variable to detect concentrations of studied parameters were analyzed with receiver operating characteristic (ROC) curves. Time had the least area under the ROC curve for TRAIL, equal to 0.891, with a sensitivity and specificity of 86.5% and 72.8%, respectively. For TRAIL-R1 area under the ROC curve was 0.811, with sensitivity and specificity of 77% and 78.5%, respectively. The ROC value for TRAIL-R2 was 0.834 and 79% sensitivity and 77% specificity.

Discussion

In spite of the variety of diagnostic methods and progress in knowledge of ovarian cancer biology, its early detection is still highly unsatisfactory. Disorders of the immune system, in particular

Table I. Concentrations of sTRAIL, sTRAIL-R1 and sTRAIL-R2 in serum of women from study and control group

Group	*N*	sTRAIL [pg/ml]		sTRAIL-R1 [ng/ml]		sTRAIL-R2 [pg/ml]	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Ovarian serous cystadenoma	35	1000.77–2096.23	1711.74 ±306.09[1,2]	10.69–36.61	21.59 ±7.66[1,3]	56.40–84.69	72.17 ±7.20[1,5]
Ovarian mature teratoma	15	1705.22–2480.62	2031.93 ±263.38[1,2]	15.59–47.24	31.09 ±9.96[1,4]	62.19–74.40	68.16 ±4.10[1,3]
Serous cystadenocarcinoma	35	2561.40–727.44	3136.53 ±289.52[1]	14.55–47.36	26.65 ±8.77[1]	59.20–73.97	67.87 ±4.42[1]
Control	30	435.52–986.33	696.67 ±173.67	0	0	0	0

Values are means ± SD. Values of studied parameters between groups were compared by Student's t-test. [1]p < 0.0001 compared to control group; [2]p < 0.0001 compared to ovarian cancer; [3]NS compared to ovarian cancer; [4]p < 0.01 NS compared to ovarian cancer; [5]p < 0.001 compared to ovarian cancer.

systemic and local immunosuppression, play an important role in the pathogenesis of this disease. The proliferative activity of neoplasms can be estimated by many methods; for example, argyrophilic nucleolar organizer regions (AgNORs) is one of them [11]. Similarly, there are many methods of assessing apoptotic activity. It is connected, to a large extent, with the increased concentration of cytokine molecules in body fluids and apoptosis disorders. A particular role in the induction of this process falls to TRAIL proteins and their receptors. The role of their soluble forms is also hardly known. Therefore, as part of our research, the concentration of sTRAIL and its receptors sTRAIL-R1 and sTRAIL-R2 was determined in the serum of women with ovarian cancer. In women from the study group the concentration of sTRAIL was higher than in healthy women. The highest concentration of the studied parameter was found in women with ovarian cancer, and it was significantly higher than its concentration in women from the control group and with a benign neoplasm, which is probably proof of autocrine secretion of this cytokine in the mechanism involving soluble agents.

So far, only a few studies aimed at determining the concentration of sTRAIL and its receptors in ovarian cancers have been conducted. Gasowska-Bodnar *et al.* [12] evaluated the kinetics of changes in serum TRAIL levels as a potential predictive and prognostic factor in patients with epithelial ovarian cancer in FIGO stage IIIC and IV. Opposite to our results, TRAIL concentration did not differ significantly between patients with ovarian cancer and the control group. Serum TRAIL level increased after two courses of neoadjuvant chemotherapy based on a paclitaxel and platinum analog. However, from the results of this study it seems that the concentration of TRAIL has no critical value as a predictive or prognostic factor in patients with ovarian cancer.

Most research on TRAIL and its receptor system has evaluated other types of cancers or autoimmune disorders. Research was conducted by Krieg *et al.* [13], who proved the increased concentration of sTRAIL ligand in serum of 41 patients with gastric carcinomas. According to the authors, depending on the type of neoplasm, different types of TRAIL ligand are detected. This selective toxicity raises great hopes for being used in diagnostics and therapy. So far, research on different cell lines has been performed. Corsten *et al.* [14] conducted research on the U87 and A172 cell lines of human glioma. Soluble TRAIL ligand in different concentrations was added to the cell growth medium. The authors demonstrated a decrease in cell vitality which correlated with an increase in the concentration of soluble TRAIL ligand. Similar observations were made by Toiyama *et al.* [15], who found that there may be a link between the concentration of TRAIL ligand in the serum and prognosis in cancer patients. They found that the concentration of sTRAIL in the serum in 84 renal cell carcinoma patients is lower than in healthy individuals. A decrease in the concentration of TRAIL ligand in the serum involved distant metastases, whereas after surgical cytoreduction of the tumor mass, the concentration of the studied parameter increased. Also Allen *et al.* [16] performed research on the role of agents involved in the regulation of the TRAIL system in the neoplastic process. In the serum of oncologic patients the researchers measured the substance TIC 10 (TRAIL-inducing compound 10) influencing TRAIL concentration. Both TRAIL ligand and TIC 10 occurred in an increased concentration in neoplasm patients. According to the researchers, TRAIL plays an important role in inhibiting the proliferation of neoplastic cells. The research of Cuello *et al.* [17], who assessed the influence of a TRAIL molecule on neoplastic cells' chemoresistance, provides interesting observations. They found that after administering the ligand, tumor growth was inhibited, which has a significant impact on the process of neoplastic lesion treatment. Qiu *et al.* [18] assessed the influence of various pro-apoptotic ligands on the U87-MG cell lines. The authors proved that in glioma the resistance to TRAIL-induced extrinsic apoptosis most frequently occurs. Szliszka *et al.* [19] suggest that TRAIL ligand may be applied in oncologic therapy. The researchers demonstrated, however, that some cell lines may be resistant to TRAIL-induced apoptosis. The research performed on three bladder cancer cell lines showed that TRAIL ligand induced apoptosis only in the SW 780 cell line, whereas the 674 47 V and T24 cell lines did not respond to TRAIL ligand. A very important element of the TRAIL system apart from the ligand is its receptors possessing an intracellular domain essential for transducing an apoptosis signal: TRAIL-R1 and TRAIL-R2.

Our research demonstrated that in women from the control group the concentrations of both receptors were below test sensitivity. The highest concentration of TRAIL-R1 was found in women with ovarian teratoma, and in comparison to women with ovarian serous cystadenocarcinoma the difference was not statistically significant. The concentration value of TRAIL-R2 was the highest in women with serous cyst adenoma, which is proof of significant involvement of these cytokines in systemic disorders of the immune system. Determination of the studied parameter concentrations may prove to be useful in differential diagnostics of benign ovarian neoplasms. No research on concentration assessment has been performed so far. However, Daniels *et al.* [20] assessed the expression of TRAIL ligand receptors in normal tissues and cancerously changed ones. It was found that the expression of TRAIL-R1 and TRAIL-R2 increases significantly

in the majority of neoplastic tumors. Apart from the above-mentioned receptors, the researchers demonstrated the statistically significantly lower expression of TRAIL-R3 and TRAIL-R4 receptors in both normal and neoplastic tissues. It is connected with the fact that TRAIL-R3 and TRAIL-R4 receptors do not play a substantial role in TRAIL-induced cytotoxicity. Similar research was conducted by Szliszka *et al.* [19]. The authors proved that an increase in TRAIL expression in the cells of TRAIL-R1 and TRAIL-R2 pro-apoptotic receptors may enhance neoplasm sensitivity to apoptosis.

One of the new promising anti-cancer therapies is TRAIL. The initial enthusiasm for TRAIL has been hampered by accumulating data demonstrating TRAIL resistance in various tumor types including ovarian cancer cells. There is, therefore, a need to identify markers of TRAIL resistance, which could represent new hits for targeted therapy that will enhance TRAIL efficacy. In addition, the identification of patients who are more likely to respond to TRAIL therapy would be highly desirable. In this review, we discuss the different molecular and cellular mechanisms leading to TRAIL resistance in ovarian cancer. In particular, we address the mechanisms involved in intrinsic, acquired and environment-mediated TRAIL resistance, and their potential implications for the clinical outcome [21].

In conclusion, the presented data can provide new information about the important role of the TRAIL-TRAIL-R system, particularly soluble factors in pathogenesis of ovarian cancer. The obtained results prove that: 1) The state of deep immunosuppression accompanying neoplastic disease depends, to a large extent, on the extrinsic pathway of apoptosis induction in the TRAIL/TRAIL-R system. 2) Particularly intensified changes in the concentration of sTRAIL in women with ovarian cancer may be proof of autocrine secretion of this cytokine in the mechanism involving soluble agents. 3) Determination of TRAIL-R1 and TRAIL-R2 concentrations may prove to be useful in differential diagnostics of ovarian neoplasms, which requires further research.

Conflict of interest

The authors declare no conflict of interest.

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