

Mesenchymal stem cells derived from adipose tissue and Ishikawa cells co-culture highlight the role of adiponectin in endometrial cancer pathogenesis

RĂZVAN CIORTEA^{1,2)}, SERGIU ȘUȘMAN^{3,4)}, ANDREI MIHAI MĂLUȚAN^{1,2)}, COSTIN BERCEANU⁵⁾, RADU FLORIN MOCAN-HOGNOGI^{1,2)}, CARMEN ELENA BUCURI^{1,2)}, OLGA SORIȚĂU⁶⁾, IOANA NEAGOE⁷⁾, DAN MIHU^{1,2)}

¹⁾"Dominic Stanca" Clinic of Obstetrics and Gynecology, Cluj-Napoca, Romania

²⁾Discipline of Obstetrics and Gynecology, Department IX of Mother and Child, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

³⁾Discipline of Histology, Department I of Morphological Sciences, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁴⁾Department of Pathology, Imogen Medical Research Institute, Cluj-Napoca, Romania

⁵⁾Discipline of Obstetrics and Gynecology, Department of Mother and Child, University of Medicine and Pharmacy of Craiova, Romania

⁶⁾Laboratory of Radiotherapy, Radiobiology and Tumor Biology, "Prof. Dr. Ion Chiricuță" Institute of Oncology, Cluj-Napoca, Romania

⁷⁾Research Center for Functional Genomics, Biomedicine and Translational Medicine, Faculty of Pharmacy, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Visceral obesity is a risk factor for endometrial cancer (EC). Visceral adipose tissue secretes over 50 inflammatory cytokines that can act centrally to regulate different physiological processes of the body but also remotely involved in communicating messages from the adipose tissue to other target tissues. The purpose of this study is to demonstrate the effect of *in vitro* adipose mesenchymal stem cells (MSCs) on endometrial tumor cells. **Materials and Methods:** Adipose-derived stem cells (ASCs) were isolated from normal subcutaneous (SC) and omentum adipose tissue from one woman without any other pathologies associated during a Fallopian tube ligature intervention. From one patient with EC was also harvested both SC and omentum adipose tissue. Ishikawa cells were cultured in ASCs conditioned medium. Study outcomes included detection of adipokines in cell culture supernatants and cell lysates by the enzyme-linked immunosorbent assay (ELISA). **Results:** Our results indicate that cells from the EC patient's fat tissues migrated during the first days of cultivation and had a high proliferation rate. Ishikawa cells grown in MSCs co-culture showed lower absolute values of adiponectin than the cells cultured individually, having a pro-tumoral effect. The differences were statistically significant compared to Ishikawa cells in monoculture. In supernatants of MSCs, an increase in adiponectin's values in MSCs from SC adipose tissue of the patient with EC (SC cMSCs) was observed in co-culture as compared to monocellular control culture. **Conclusions:** Our data confirm the hypothesis that ASCs are an important source of intracellular adiponectin, which increase the EC cell proliferation.

Keywords: adipose tissue, mesenchymal stem cells, adiponectin, endometrial cancer.

Introduction

Endometrial cancer (EC) is associated with obesity in 40% of cases [1]. The hypothesis of the stimulatory effect of adipocyte secretion products on the endometrium is used to explain obesity and EC [2]. This hypothesis cannot, however, explain the effects on the entire population since not all obese women develop EC. One of the possible explanations may be the variability of the secretory profile of adipocyte in terms of pro- and antitumoral factors.

Recent studies have highlighted the interrelationship between tumor cells and other tissues in the body [3]. These can be located either near the tumor, in the stroma (cancer-associated fibroblasts [4], immune cells) or at a distance from it (adipose cells or other endocrine structures). This dialogue performed by circulating molecules can remotely influence the biology of the tumor cell by modifying the secretory profile [5].

Adipose tissue contains a population of mesenchymal stem cells (MSCs), which can give rise to fully differentiated fat cells [6]. Also, adipose-derived stem cells (ASCs) can be differentiated into osteogenic, chondrogenic, muscular or neuronal cell line [7–9]. ASCs and derived cells can secrete growth factors: vascular endothelial growth factor-A (VEGF-A), epithelial growth factor (EGF), insulin growth factor (IGF), cytokines (leptin, adiponectin, omentin), inflammatory factors – tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and interleukin-8 (IL-8) with pro- or antitumoral effects [10].

Adiponectin is an adipokine, secreted almost exclusively by the adipocyte, which has a protective role in the appearance and evolution of malignancies associated with obesity. Low serum levels are associated with EC [11], breast cancer [12], colorectal cancer [13], gastric cancer [14], prostate cancer [15] and leukemia [16]. The role of

adiponectin in EC carcinogenesis is also highlighted by a reduced plasma level in a range of other diseases: obesity, insulin resistance, type 2 diabetes, which constitute independent risk factors for EC [17]. The increased plasma level of adiponectin was associated with a 20–90% reduction in risk for EC in young and premenopausal patients [18].

The low serum level of adiponectin justifies the association with EC through specific mechanisms through which increase of the bioavailable forms of estrogens, enhancement of IGF-1 bioactivity, increase of the production of ovarian sex steroids [19] take place. Adiponectin also exerts antitumoral effect by: inhibiting angiogenesis both *in vivo* and *in vitro* [20] and macrophage activity [21], selective sequestration of growth factors at the pre-receptor level by inhibiting their binding to membrane receptors [22], activation of some signal molecules, such as: pyruvate kinase (PK), nuclear factor-kappaB (NF- κ B), peroxisome proliferator-activated receptor (PPAR), mitogen-activated protein kinase (MAPK) with known implications in carcinogenesis [22, 23].

The evidence accumulated so far indicates that the dosage of adiponectin can be a useful screening method; a low level is useful in the early detection of EC linked to obesity. Even more, adiponectin or its homologues may be antineoplastic agents and may have important therapeutic implications. The purpose of this study is to demonstrate the *in vitro* effect of adiponectin secreted by ASCs on endometrial tumor cells in order to improve the management of obese patients at risk for developing EC.

Materials and Methods

Harvesting the adipose tissue from subcutaneous tissue and from the omentum

Normal adipose tissues were surgically removed from the subcutaneous (SC) sites (abdominal wall) and from the omentum during surgery. The fragments were immersed in complete Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F-12) with 10% fetal bovine serum (FBS) and transported to the laboratory. The informed consent was obtained from the patient, the form delivered to the patient being approved by the Ethical Committee of the "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania (Approval No. 305/29.06.2016).

Cell isolation from adipose tissues

Fragments from SC tissue and omentum were placed in 50 mL Falcon tubes containing 10 mL of complete medium [DMEM/F-12 (1:1, v/v)], 10% FBS, 2 mM L-Glutamine, 1% non-essential amino acids (NEA) and 1% Penicillin–Streptomycin. Fragments were washed with phosphate-buffered saline (PBS) and transferred in small Petri dish containing 1 mL complete medium. Tissues were undergone to mechanical action, cut into small pieces and incubated for 30 minutes in 0.1% collagenase IV (Gibco) in an orbital rotating incubator. After incubation, the suspension was centrifuged at 1300 rpm for 5 minutes. Cells pellet was transferred into 25-cm² culture flasks (Nunc) containing 7 mL complete standard stem cell

medium (DMEM/F-12, 15% FBS, 2 mM L-Glutamine, 1% NEA, 1 mM Sodium Pyruvate, 55 μ M β -Mercapto-ethanol and 1% Penicillin–Streptomycin – all reagents from Sigma-Aldrich) and incubated in a humidified 5% CO₂ atmosphere. The first cells adhered after 2–7 days and medium was changed at 2–3 days until they reached the confluence.

Normal SC and omentum adipose tissue was harvested from women without any other associated pathology, during a fallopian tube ligature intervention. From the patient with EC, endometrioid type, both SC tissue and omental adipose tissues were harvested.

Ishikawa cells cultures

Ishikawa cell line was purchased from American Type Culture Collection (ATCC) cell bank. The cultivation medium consisted of MEM medium with 10% FBS, 2 mM L-Glutamine, 1 mM Sodium Pyruvate, 1% NEA, and 1% antibiotics. Cells presented a relative high proliferation rate; therefore, they were split every 2–3 days.

Flow cytometry analysis

After the sixth passage, when the isolated cells reached 80–90% confluence, cells were prepared for immunophenotyping by flow cytometry. Medium was removed, and cells were washed with PBS 1X (pH 7.2), trypsinized with 0.25% Trypsin and were incubated 5 minutes at 37°C, followed by trypsin inactivation with 5 mL of complete medium, collected into a 15 mL tube and centrifuged at 1100 rpm for 5 minutes. For each marker were added 10⁵ cells per tube. The primary conjugated antibodies dilution was 1:20 for BD antibodies [cluster of differentiation (CD) 29 Phycoerythrin (PE), CD34 Fluorescein Isothiocyanate (FITC), CD45 FITC, CD49e PE] (BD Biosciences, San Jose, CA, USA) and 1:50 dilution for Santa Cruz Biotechnologies antibodies (CD73 PE, CD90 FITC, CD105 PE, CD117 PE). Cells were incubated for 45 minutes at 4°C in the dark. After incubation, cells were washed twice with ice cold PBS 1X and then analyzed by flow cytometry (BD FACS Canto II flow cytometer; Becton Dickinson, USA) using Diva software.

Co-culture protocol

MSCs and Ishikawa cells were co-cultivated using 0.4 μ m membrane pores inserts, so only the exchange of cytokines and other biomolecules took place. We used Millicell® Cell Culture Inserts for plates with 24 wells. MSCs were seeded into 24 well (10⁵ cells in 800- μ L stem cell medium). Ishikawa cells were cultivated into inserts (10⁵ cells in 500- μ L MEM complete medium). Control cells were referred to as follows: Ishikawa, MSCs from normal SC adipose tissue (SC nMSCs, omentum nMSCs), MSCs from SC adipose tissue of the patient with EC (SC cMSCs, omentum cMSCs). Besides the co-cultures, the isolated cells of each line and Ishikawa cells were grown under the same conditions (number of cells, amount of medium). After 48 hours of co-cultivation, the supernatant from each co-culture (MSCs and Ishikawa) was collected. The monocellular layers were lysed using CellLytic™ M Cell Lysis Reagent, suitable for mammalian cell lysis and protein solubilization (Sigma-Aldrich). The monocultures were treated in a similar manner. Both

supernatants and lysates were frozen and stored in freezer at -80°C until the probes were analyzed for adiponectin content through enzyme-linked immunosorbent assay (ELISA) technique.

Detection of adipokines in cell culture supernatants and cell lysates by ELISA

For adiponectin detection, we used Human Adiponectin ELISA Kit (Abbexa Ltd., Innovation Centre, Cambridge Science Park, Cambridge, CB4 0EY, UK) with test range between 1.56–100 ng/mL. Standards and samples were added undiluted into the wells of pre-coated plate (100 μL /well) in duplicate. The plate was incubated at 37°C for 90 minutes, followed by adding Biotin-conjugated antibody. The plate was incubated for 60 minutes at 37°C and washed three times with an automated washer. Avidin–Biotin–peroxidase complex solution was added with an incubation of 30 minutes at 37°C . After washing the plate five times, 3,3',5,5'-Tetramethylbenzidine (TMB) substrate was added and the plate was incubated for 30 minutes in dark at 37°C . The development of color was stopped and the plate was read with a Sunrise Tecan microplate reader using Magellan 3 software for extrapolation and calculation of results.

Statistical analysis

All experiments were performed in duplicate using ELISA tests and expressed as concentrations extrapolated from standard curves. Statistical analyses were performed

using Prism version 5.0 (GraphPad, San Diego, CA, USA). Statistical significance between groups was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison *post-hoc* test and expressed as (*) when $p < 0.05$, (**) when $p < 0.01$ and (***) when $p < 0.001$, or by Student's *t*-test with Welch's correction.

Results

Isolation of MSCs

Obtaining a primary culture from SC tissue or from omentum of patients without cancer pathology *versus* adipose tissues from the EC patient was different. The cells of normal SC tissue migrated later and reached to confluence in about three weeks when the first passage (split) was performed. Cells from the EC patients' fat tissues migrated during the first days of cultivation and had a high proliferation rate. We can see from our results a difference regarding the migration potential between the normal adipose tissue and adipose tissue harvested from patients with EC. This could suggest a pro-proliferative status due to the presence of the tumor in the body and a possible dialogue between the tumor and other cells. Isolated cells exhibited a characteristic fibroblastoid-like morphology, especially after the third passage. The proliferation rate and the morphology were maintained even at more advanced passages without sign of senescence (Figure 1).

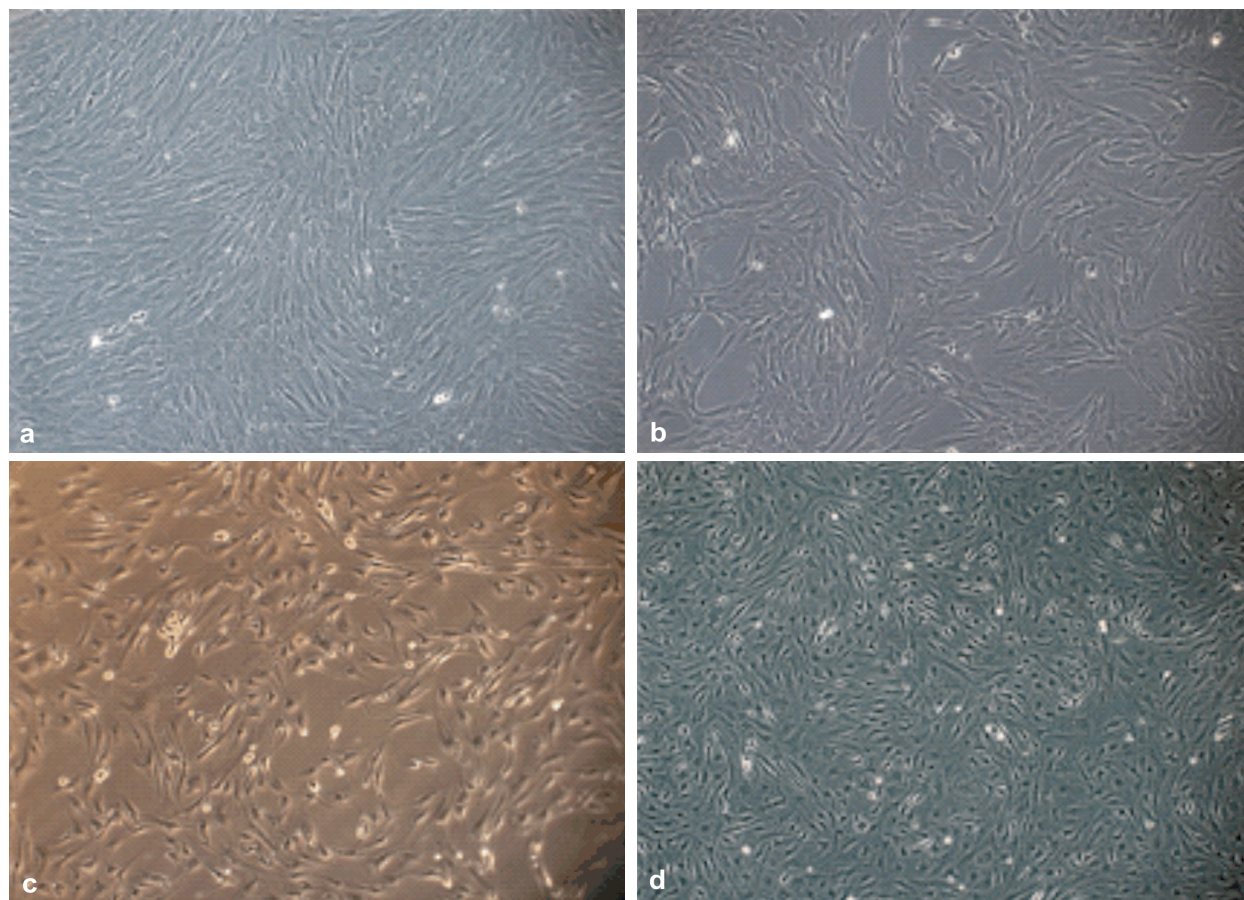


Figure 1 – Optical microscopy images ($\times 100$) of isolated cells from fat tissues (SC tissue and omentum) of patients with EC or without EC at passage 6: (a) MSCs from SC tissue, normal subject; (b) MSCs from SC tissue of patient with EC; (c) MSCs from normal omentum; (d) MSCs from EC omentum. SC: Subcutaneous; EC: Endometrial cancer; MSCs: Mesenchymal stem cells.

Characterization of MSCs by flow cytometry

The expression of MSCs markers was investigated by flow cytometry. The isolated cells expressed characteristic markers of MSCs: negativity for CD34, CD45 and CD117, high positivity for CD29, CD49e, CD73, CD90, CD105, CD271. The profile of the cells isolated allows us to perform the experiments being concordant with the classical profile of stem cells isolated from the adipose tissue. An interesting exception was the negativity for CD105 in the case of MSCs isolated from normal omentum (Table 1, Figure 2).

Table 1 – The expression level of characteristic antigens of MSCs by flow cytometry

Antibody	SC nMSCs % of events	SC cMSCs % of events	Omentum nMSCs % of events	Omentum cMSCs % of events
CD29-PE (+)	98.6	99	99.8	100
CD34-FITC (-)	2.7	3.5	2.6	3.3
CD45-FITC (-)	2.6	3.8	2.7	2.8
CD49e-PE (+)	97.4	99.2	98.9	99.5
CD73-PE (+)	99.5	99.6	99.6	100
CD90-FITC (+)	98.7	97.9	96.9	99.4
CD105-PE (+)	97.8	91.2	3.4	98.7
CD117-PE (-)	1.6	3.3	0.4	2.2

MSCs: Mesenchymal stem cells; SC: Subcutaneous; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with endometrial cancer; CD: Cluster of differentiation; PE: Phycoerythrin; FITC: Fluorescein Isothiocyanate.

Adiponectin expression of Ishikawa cells and MSCs in co-culture supernatants and cell lysates

Values of adiponectin varied between 4.89 and 2.5 ng/10⁵ cells. Lysates obtained from Ishikawa control cells showed the highest values, up to 4.89 ng. In supernatants, the values of Ishikawa's cells averaged 2.7 ng, almost reaching the others type of cells. A difference was observed between cellular lysates of MSCs; lysate of omentum cMSC showed higher values than the rest of MSCs lysates. Supernatants did not show statistically significant differences (Figure 3).

Co-cultures lysates

Ishikawa cells grown in MSCs co-culture showed lower absolute values than the cells cultured individually: 4.56 ng (co-culture with SC nMSCs) and 3.53 ng (co-culture with SC cMSCs). The differences were statistically significant as compared to Ishikawa cells in monoculture (Figure 4). These results highlight that in our co-culture model MSCs isolated from the adipose tissue are able to stimulate the proliferation of Ishikawa cells.

Co-cultures supernatants

Adiponectin values in Ishikawa cells supernatants were similar to those of the Ishikawa cells monocellular culture supernatant. The highest values were observed in the supernatant from co-culture with cMSCs omentum. There were no statistically significant differences (Figure 5).

In supernatants of MSCs, an increase in adiponectin values in SC cMSCs was observed in co-culture compared to monocellular control culture. A different situation was

in the case of nMSC omentum, where adiponectin concentration decreased in the supernatant of co-culture with Ishikawa cells (Figure 6).

Discussions

Starting from the idea that in modern society the prevalence of obesity is rising and that the adipose tissue is directly correlated with a range of conditions, the dysfunction of adipose tissue is today referred as an individual pathological entity [24]. Nowadays, adipose tissue is not considered to be an energy storage organ anymore, but an endocrine organ, with a secretory potential insufficiently characterized [25]. The adipocyte synthesizes and releases into circulation substances that can act at the central level where they regulate the physiological processes of the body. Adipocyte biomolecules (leptin, adiponectin) have been identified to play a role in communicating messages from the adipose tissue to other target tissues [26]. Obesity induces a change in body fat distribution (intraabdominal fat) with consequences on cytokine secretion, chemokines, and immune cell distribution [27].

The cells isolated from the adipose tissue (SC and omentum) have a characteristic profile for MSCs, being positive for CD29, CD49e, CD73, CD90, CD105, CD271 and negative for CD34, CD45 and CD117. There is a characteristic profile for other types of MSCs, such as those isolated from the bone marrow that also express CD29 and CD90 [28]. CD90 (Thy-1) is an adhesion molecule specifically present at MSCs level, having a role in cell-cell and cell-extracellular matrix interaction, being involved in cellular migration [29]. CD105 (endoglin) is involved in cell proliferation and differentiation [30]. Its positivity is maintained during differentiation, and it is present in the MSCs expressing CD105 that can be differentiated to the adipogenic, chondrogenic and osteogenic line [31, 32]. Our finding, CD271, a cluster of positive differentiation in the isolated cells, has been proposed as a versatile marker for isolation of multipotent stem cells from different sources, except umbilical cord blood and Wharton's jelly [33, 34]. CD271 is a suitable marker for isolating stem cells from the adipose tissue in order to use tissue regeneration and autologous cell transplantation [35]. However, the use of mesenchymal cells derived from adipose tissue (ASCs) in cell therapy should be made with caution given that protumoral effects have been reported in breast cancer [36]. Negativity for CD34 and CD45 excludes the presence of endothelial or hematopoietic cells [37].

In this study, we have attempted to highlight some of the molecular mechanisms by which endometrial tumors, endometrioid type, interact with MSCs in adipose tissue. For these, we have created an *in vitro* experimental model that has allowed us to show that ASCs have a stimulating effect on endometrial tumor cells. We also used Ishikawa cells as a cell line commonly used in literature studies, trying to avoid the unpredictable biology that could have been generated by tumor cells from patients with heterogeneous anthropometric and biological characteristics.

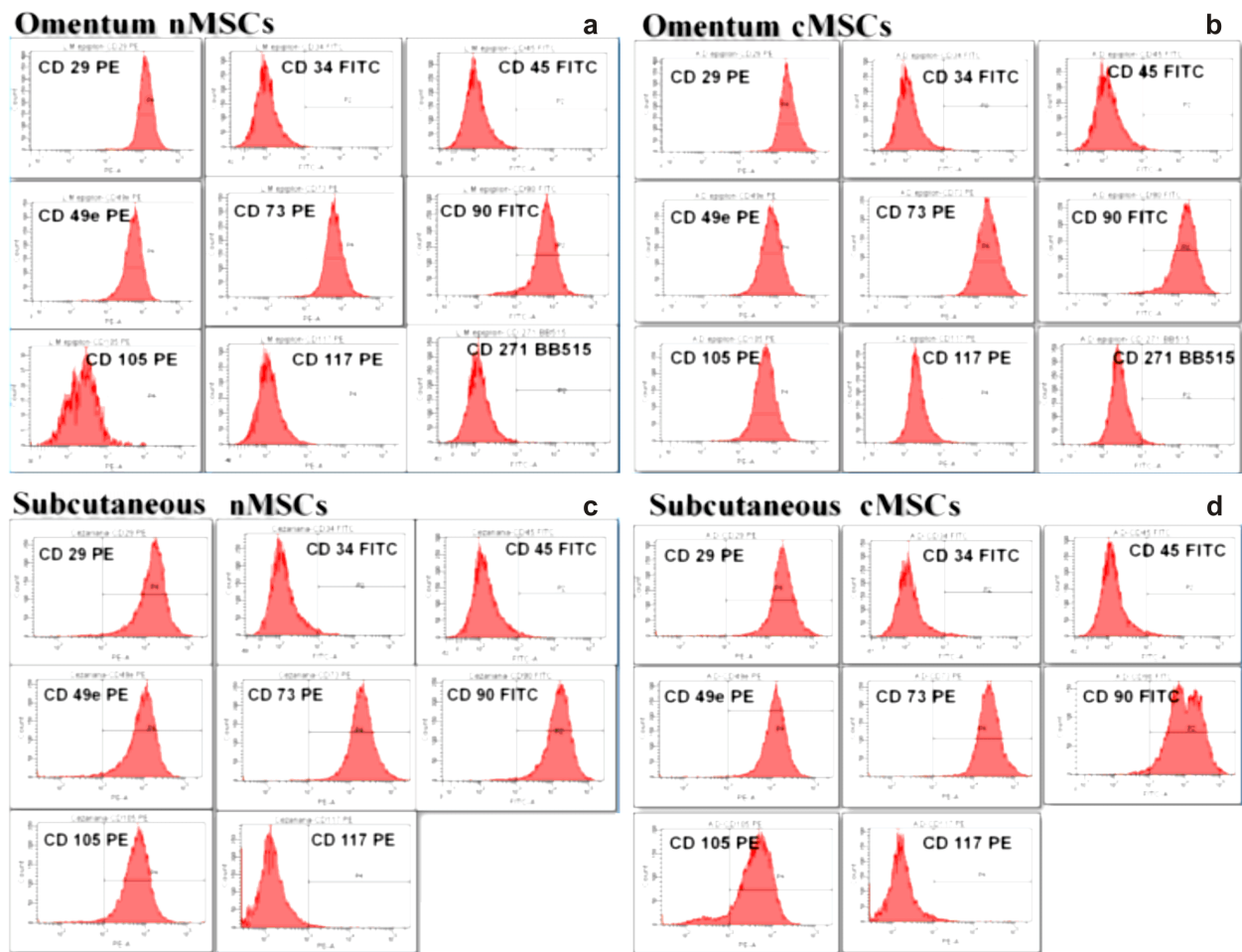


Figure 2 – (a–d) Histograms from flow cytometry analysis of isolated cells from fat tissues (SC tissue and omentum) from patients without EC (nMSCs) and with EC (cMSC). SC: Subcutaneous; EC: Endometrial cancer; MSCs: Mesenchymal stem cells; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with EC; CD: Cluster of differentiation; PE: Phycoerythrin; FITC: Fluorescein Isothiocyanate; BB515: Brilliant™ Blue 515.

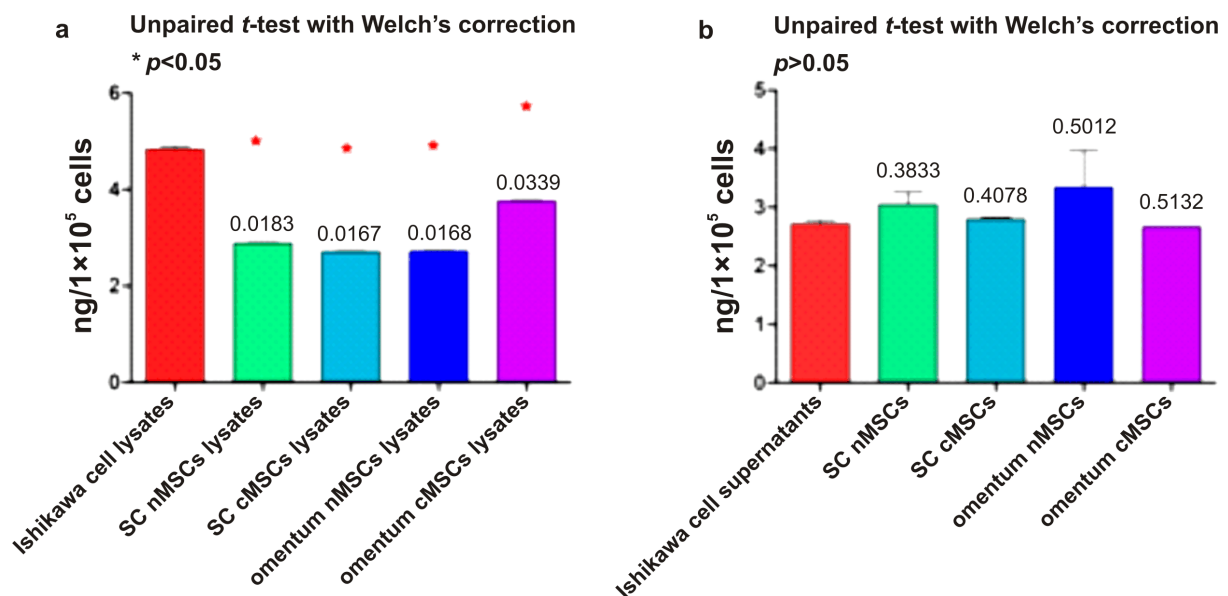


Figure 3 – Adiponectin levels evaluated by ELISA method from isolated cell cultures from lysates (a) and supernatants (b). ELISA: Enzyme-linked immunosorbent assay; SC: Subcutaneous; MSCs: Mesenchymal stem cells; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with endometrial cancer.

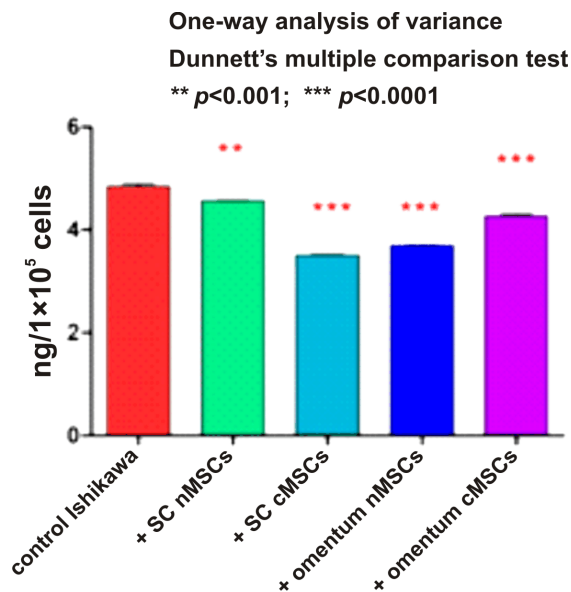


Figure 4 – Adiponectin levels evaluated by ELISA method co-cultures of Ishikawa cells and MSCs from cell lysates. ELISA: Enzyme-linked immunosorbent assay; MSCs: Mesenchymal stem cells; SC: Subcutaneous; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with endometrial cancer.

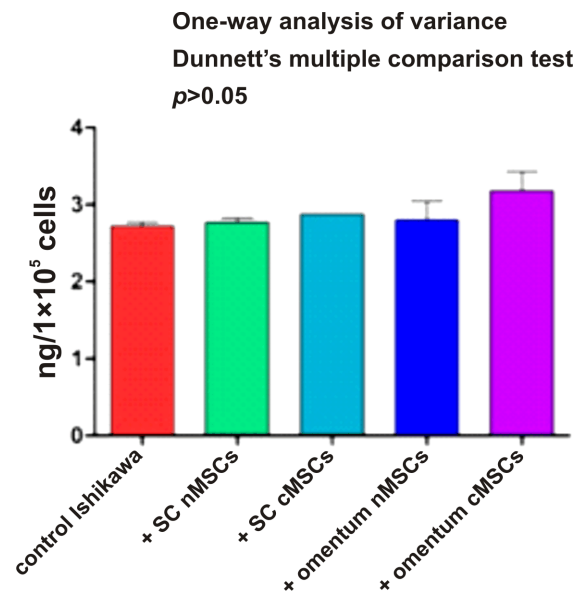


Figure 5 – Adiponectin levels evaluated by ELISA method co-cultures of Ishikawa cells and MSCs from Ishikawa culture supernatants. ELISA: Enzyme-linked immunosorbent assay; MSCs: Mesenchymal stem cells; SC: Subcutaneous; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with endometrial cancer.

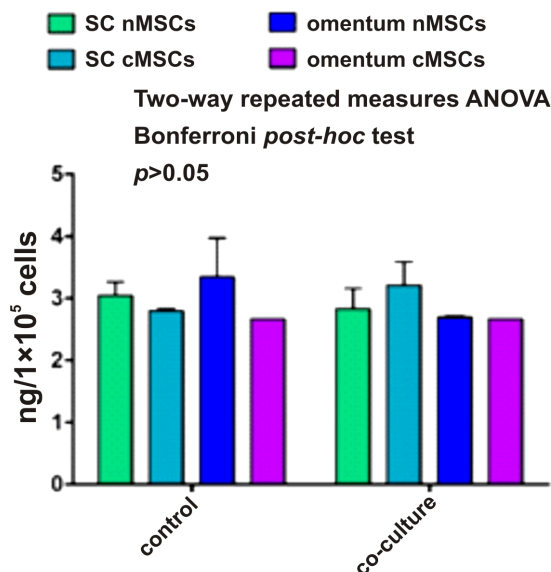


Figure 6 – Adiponectin levels evaluated by ELISA method co-cultures of Ishikawa cells and MSCs from MSCs culture supernatants. ELISA: Enzyme-linked immunosorbent assay; MSCs: Mesenchymal stem cells; SC: Subcutaneous; nMSCs: MSCs from normal SC adipose tissue; cMSCs: MSCs from SC adipose tissue of the patient with endometrial cancer; ANOVA: Analysis of variance.

In our study, co-cultivation of endometrial tumor cells (Ishikawa line) with ASCs resulted in a decrease in intracellular adiponectin level in tumor cells. This demonstrates that there is a dialogue between the two cellular entities that they can change their proliferation rate and differentiation capability. Unlike most fat-derived proteins, adiponectin levels are lower in obese as compared to sub- or normoponderal subjects, although adipocytes are the most important source of adiponectin [38]. The

plasma level of adiponectin is 53% higher in the non-obese subjects as compared to the obese ones [39]. Our results show that ASCs have a stimulating effect on the endometrial tumor cell, a decrease intracellular level of adiponectin being associated with proliferative status. These findings are consistent with other studies, ASCs leading to increased chemoresistance in patients diagnosed with breast cancer [40]. Similar results have been reported by other studies that have shown the role of leptin secreted by adipose cells in stimulating the secretion of VEGF, IL-1 β , leukemia inhibitory factor (LIF) in EC cell lines [41]. In addition to the activation of local stromal cells (cancer associated fibroblasts), recruitment of ASCs by mobilization from the adipose tissue was also reported [42]. Differences between ASCs sources (omentum, SC tissue) have been reported, the omentum being an important resource for EC progression [43].

Another reason why the low level of adiponectin is associated with EC could be explained by antiangiogenic properties [19]. Adiponectin inhibits angiogenesis both *in vivo* and *in vitro* [28]. It has also been reported in the literature that adiponectin inhibits tumor growth by suppressing the development of neovessels in rats [44]. These findings support the idea that low levels of adiponectin are associated with increased angiogenesis required for the development of EC.

A low intracellular level obtained by us correlates with pro-angiogenic effects as pro-tumoral and morphological nutritional support. This was also reported by Klopp *et al.*, who have shown that adipose cells derived from the omentum stimulate vascularization in EC [45]. ASCs play a role in the adipose tissue, which functions as a stem cell reservoir [46]. Previous studies have shown a pericapillary location of migrated ASCs in tumors [47]. This fact suggests that ASCs can stimulate tumor progression through angiogenesis.

The plasma level of adiponectin is higher in women than in men, unrelated to body fat or fat distribution. Postmenopausal women have significantly lower levels compared to those in the premenopause, which justifies increased EC incidence in patients with low estrogen levels [48]. These data suggest another mechanism by which adiponectin would favor the development of EC, the change in estro-progestational balance being known as a risk factor in this pathological entity. Another mechanism by which low serum levels of adiponectin alters the estro-progestational balance is the decrease in fatty acid oxidation in the skeletal muscle but also the increase in hepatic glucose synthesis. Secondary hyperglycemia occurs with insulin resistance and hyperinsulinism. Increased insulin inhibits sex hormone binding globulin (SHBG) and IGF-binding protein-1 (IGFBP-1), thereby increasing bioavailable forms of estrogen but also the bioavailability of IGF-1 [17]. Data published in the literature consider that insulin increase the level of sex steroids secreted by the ovary [19]. Exposure to an elevated estrogenic level that is not counterbalanced by progesterone is the dominant hypothesis in the etiopathogenesis of EC.

Adiponectin may activate MAPK, which in turn activates endothelial nitric oxide (NO) synthase, thereby increasing NO production [49]. MAPK phosphorylates and activates tuberous sclerosis protein 2 (TSC2), a tumor suppressor that inhibits protein synthesis [23]. Thus, activation of MAPK by adiponectin may also justify its involvement in tumor proliferation.

There are multiple mechanisms that explain the adiponectin–EC relationship. Dialogue between endometrial tumor cells and ASCs *via* cytokines can help explain this interaction. Our study is one of the first to highlight the *in vitro* dialogue between isolated MSCs from the adipose tissue and endometrial tumor cells (Ishikawa line) through co-cultivation experiment. Our data are in line with other studies recently published in the literature on the topic [50].

✉ Conclusions

The results obtained by us show that ASCs have an endometrial tumor cell stimulating effect, adiponectin being involved in this dialogue. These preliminary results open up a new perspective regarding the decryption of the mechanisms through which MSCs and EC interact and the information obtained may be the basis for the development of new therapies or new methods of screening and early diagnosis of EC.

Conflict of interests

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

Authors' contribution

Răzvan Ciortea and Costin Berceanu equally contributed to the manuscript.

Acknowledgments

This work was supported by “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania, Project number 4944/9/08.03.2016.

References

- [1] Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr*, 2006, 83(2):461S–465S.
- [2] Nair S, Nguyen H, Salama S, Al-Hendy A. Obesity and the endometrium: adipocyte-secreted proinflammatory TNF α cytokine enhances the proliferation of human endometrial glandular cells. *Obstet Gynecol Int*, 2013, 2013:368543.
- [3] Harper J, Sainson RC. Regulation of the anti-tumour immune response by cancer-associated fibroblasts. *Semin Cancer Biol*, 2014, 25:69–77.
- [4] Tao L, Huang G, Song H, Chen Y, Chen L. Cancer associated fibroblasts: an essential role in the tumor microenvironment. *Oncol Lett*, 2017, 14(3):2611–2620.
- [5] De Marco P, Lappano R, De Francesco EM, Cirillo F, Pupo M, Avino S, Vivacqua A, Abonante S, Picard D, Maggiolini M. GPER signalling in both cancer-associated fibroblasts and breast cancer cells mediates a feedforward IL1 β /IL1R1 response. *Sci Rep*, 2016, 6:24354.
- [6] Linkov F, Kokai L, Edwards R, Sheikh MA, Freese KE, Marra KG, Rubin JP. The role of adipose-derived stem cells in endometrial cancer proliferation. *Scand J Clin Lab Invest Suppl*, 2014, 244:54–58; discussion 57–58.
- [7] Iwen KA, Prieue AC, Winnefeld M, Rose C, Siemers F, Rohwedel J, Cakiroglu F, Lehnert H, Schepky A, Klein J, Kramer J. Gluteal and abdominal subcutaneous adipose tissue depots as stroma cell source: gluteal cells display increased adipogenic and osteogenic differentiation potentials. *Exp Dermatol*, 2014, 23(6):395–400.
- [8] Noël D, Caton D, Roche S, Bony C, Lehmann S, Casteilla L, Jorgensen C, Cousin B. Cell specific differences between human adipose-derived and mesenchymal-stromal cells despite similar differentiation potentials. *Exp Cell Res*, 2008, 314(7): 1575–1584.
- [9] Zavan B, Michelotto L, Lancerotto L, Della Puppa A, D'Avella D, Abatangelo G, Vindigni V, Cortivo R. Neural potential of a stem cell population in the adipose and cutaneous tissues. *Neurol Res*, 2010, 32(1):47–54.
- [10] Zhang Y, Daquinag A, Traktuev DO, Amaya-Manzanares F, Simmons PJ, March KL, Pasqualini R, Arap W, Kolonin MG. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. *Cancer Res*, 2009, 69(12):5259–5266.
- [11] Rose DP, Komninou D, Stephenson GD. Obesity, adipocytokines, and insulin resistance in breast cancer. *Obes Rev*, 2004, 5(3):153–165.
- [12] Sugiyama M, Takahashi H, Hosono K, Endo H, Kato S, Yoneda K, Nozaki Y, Fujita K, Yoneda M, Wada K, Nakagama H, Nakajima A. Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. *Int J Oncol*, 2009, 34(2): 339–344.
- [13] Ishikawa M, Kitayama J, Kazama S, Hiramatsu T, Hatano K, Nagawa H. Plasma adiponectin and gastric cancer. *Clin Cancer Res*, 2005, 11(2 Pt 1):466–472.
- [14] Goktas S, Yilmaz MI, Caglar K, Sonmez A, Kilic S, Bedir S. Prostate cancer and adiponectin. *Urology*, 2005, 65(6):1168–1172.
- [15] Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood*, 2000, 96(5):1723–1732.
- [16] Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev*, 2002, 11(12):1531–1543.
- [17] Petridou E, Mantzoros C, Dessypris N, Koukoulomatis P, Addy C, Voulgaris Z, Chrousos G, Trichopoulos D. Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. *J Clin Endocrinol Metab*, 2003, 88(3):993–997.
- [18] Lukanova A, Zeleniuch-Jacquotte A, Lundin E, Micheli A, Arslan AA, Rinaldi S, Muti P, Lenner P, Koenig KL, Biessy C, Krogh V, Riboli E, Shore RE, Stattin P, Berrino F, Hallmans G, Toniolo P, Kaaks R. Prediagnostic levels of C-peptide, IGF-I, IGFBP -1, -2 and -3 and risk of endometrial cancer. *Int J Cancer*, 2004, 108(2):262–268.

- [19] 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 U S A*, 2004, 101(8):2476–2481.
- [20] Dal Maso L, Augustin LSA, Karalis A, Talamini R, Franceschi S, Trichopoulos D, Mantzoros CS, La Vecchia C. Circulating adiponectin and endometrial cancer risk. *J Clin Endocrinol Metab*, 2004, 89(3):1160–1163.
- [21] Wang Y, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, Xu A. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem*, 2005, 280(18):18341–18347.
- [22] Kuhajda FP. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition*, 2000, 16(3):202–208.
- [23] Philp AJ, Campbell IG, Leet C, Vincan E, Rockman SP, Whitehead RH, Thomas RJ, Phillips WA. The phosphatidylinositol 3'-kinase *p85alpha* gene is an oncogene in human ovarian and colon tumors. *Cancer Res*, 2001, 61(20):7426–7429.
- [24] Blüher M. Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes*, 2009, 117(6):241–250.
- [25] Göddeke S, Kotzka J, Lehr S. Investigating the adipose tissue secretome: a protocol to generate high-quality samples appropriate for comprehensive proteomic profiling. *Methods Mol Biol*, 2015, 1295:43–53.
- [26] Gan L, Liu Z, Luo D, Ren Q, Wu H, Li C, Sun C. Reduced endoplasmic reticulum stress-mediated autophagy is required for leptin alleviating inflammation in adipose tissue. *Front Immunol*, 2017, 8:1507.
- [27] Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN Inflamm*, 2013, 2013:139239.
- [28] 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.
- [29] Schieker M, Pautke C, Reitz K, Hemraj I, Neth P, Mutschler W, Milz S. The use of four-colour immunofluorescence techniques to identify mesenchymal stem cells. *J Anat*, 2004, 204(2):133–139.
- [30] Aslan H, Zilberman Y, Kandel L, Liebergall M, Oskouian RJ, Gazit D, Gazit Z. Osteogenic differentiation of noncultured immunisolated bone marrow-derived CD105+ cells. *Stem Cells*, 2006, 24(7):1728–1737.
- [31] Roura S, Farré J, Soler-Botija C, Llach A, Hove-Madsen L, Cairó JJ, Gódia F, Cinca J, Bayes-Genis A. Effect of aging on the pluripotential capacity of human CD105+ mesenchymal stem cells. *Eur J Heart Fail*, 2006, 8(6):555–563.
- [32] Barry FP, Boynton RE, Haynesworth S, Murphy JM, Zaia J. The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem Biophys Res Commun*, 1999, 265(1):134–139.
- [33] Patel DM, Shah J, Srivastava AS. Therapeutic potential of mesenchymal stem cells in regenerative medicine. *Stem Cells Int*, 2013, 2013:496218.
- [34] Margossian T, Reppel L, Makdisy N, Stoltz JF, Bensoussan D, Huselstein C. Mesenchymal stem cells derived from Wharton's jelly: comparative phenotype analysis between tissue and *in vitro* expansion. *Biomed Mater Eng*, 2012, 22(4):243–254.
- [35] Cuevas-Díaz Duran R, González-Garza MT, Cardenas-Lopez A, Chavez-Castilla L, Cruz-Vega DE, Moreno-Cuevas JE. Age-related yield of adipose-derived stem cells bearing the low-affinity nerve growth factor receptor. *Stem Cells Int*, 2013, 2013:372164.
- [36] Ritter A, Friemel A, Fornoff F, Adjan M, Solbach C, Yuan J, Louwen F. Characterization of adipose-derived stem cells from subcutaneous and visceral adipose tissues and their function in breast cancer cells. *Oncotarget*, 2015, 6(33):34475–34493.
- [37] Huizer K, Mustafa DAM, Spelt JC, Kros JM, Sacchetti A. Improving the characterization of endothelial progenitor cell subsets by an optimized FACS protocol. *PLoS One*, 2017, 12(9):e0184895.
- [38] Beltowski J. Adiponectin and resistin – new hormones of white adipose tissue. *Med Sci Monit*, 2003, 9(2):RA55–RA61.
- [39] Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: *in vivo* and *in vitro* investigations in humans. *Am J Physiol Endocrinol Metab*, 2003, 285(3):E527–E533.
- [40] Eterno V, Zambelli A, Pavesi L, Villani L, Zanini V, Petrolo G, Manera S, Tuscano A, Amato A. Adipose-derived mesenchymal stem cells (ASCs) may favour breast cancer recurrence via HGF/c-Met signaling. *Oncotarget*, 2014, 5(3):613–633.
- [41] Carino C, Olawaiye AB, Cherfils S, Serikawa T, Lynch MP, Rueda BR, Gonzalez RR. Leptin regulation of proangiogenic molecules in benign and cancerous endometrial cells. *Int J Cancer*, 2008, 123(12):2782–2790.
- [42] Strong AL, Burow ME, Gimble JM, Bunnell BA. Concise review: The obesity cancer paradigm: exploration of the interactions and crosstalk with adipose stem cells. *Stem Cells*, 2015, 33(2):318–326.
- [43] Shah FS, Li J, Dietrich M, Wu X, Hausmann MG, LeBlanc KA, Wade JW, Gimble JM. Comparison of stromal/stem cells isolated from human omental and subcutaneous adipose depots: differentiation and immunophenotypic characterization. *Cells Tissues Organs*, 2014, 200(3–4):204–211.
- [44] Huang CY, Chang AC, Chen HT, Wang SW, Lo YS, Tang CH. Adiponectin promotes VEGF-C-dependent lymphangiogenesis by inhibiting miR-27b through a CaMKII/AMPK/p38 signaling pathway in human chondrosarcoma cells. *Clin Sci (Lond)*, 2016, 130(17):1523–1533.
- [45] Klopp AH, Zhang Y, Solley T, Amaya-Manzanera F, Marini F, Andreff M, Debeb B, Woodward W, Schmandt R, Broaddus R, Lu K, Kolonin MG. Omental adipose tissue-derived stromal cells promote vascularization and growth of endometrial tumors. *Clin Cancer Res*, 2012, 18(3):771–782.
- [46] Natesan S, Zhang G, Baer DG, Walters TJ, Christy RJ, Suggs LJ. A bilayer construct controls adipose-derived stem cell differentiation into endothelial cells and pericytes without growth factor stimulation. *Tissue Eng Part A*, 2011, 17(7–8):941–953.
- [47] Rowan BG, Gimble JM, Sheng M, Anbalagan M, Jones RK, Frazier TP, Asher M, Lacayo EA, Friedlander PL, Kutner R, Chiu ES. Human adipose tissue-derived stromal/stem cells promote migration and early metastasis of triple negative breast cancer xenografts. *PLoS One*, 2014, 9(2):e89595.
- [48] Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*, 2003, 46(4):459–469.
- [49] Goldstein BJ, Scalia R. Adiponectin: a novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab*, 2004, 89(6):2563–2568.
- [50] Li M, Li X, Zhao L, Zhou J, Cheng Y, Wang J, Wei L. Human omental adipose-derived stem cells from donors with different body mass index had similar effects on proliferation and migration of endometrial cancer cells *in vitro*. *J Obstet Gynaecol Res*, 2018, Oct 1.

Corresponding author

Sergiu Șuşman, Associate Professor, MD, PhD, Discipline of Histology, Department I of Morphological Sciences, "Iuliu Hațieganu" University of Medicine and Pharmacy, 6–8 Louis Pasteur Street, 400349 Cluj-Napoca, Cluj County, Romania; Phone +40747–074 018, e-mail: serman_s@yahoo.com