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RESEARCH ARTICLE

Evaluation of Changes in the Expression Pattern of EDIL3 in Different Grades of Endometrial Cancer

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Abstract: ***Background***: EDIL3 is an extracellular matrix protein that plays a key role in angiogenesis. Changes in the pattern of its expression also affect cellular processes and the tumor microenvironment. Elevated level of EDIL3 is considered an unfavorable prognostic marker of survival.

Objective: The aim of this study was to evaluate the changes in EDIL3 expression in endometrial cancer at various degrees of its differentiation (G1-G3) and to discuss its potential role as a molecular diagnostic marker and therapeutic target.

Methods: The study group consisted of 45 patients with endometrial cancer: G1, 17; G2, 15; G3, 13. The control group (C) included 15 patients without neoplastic changes. The expression of EDIL3 was assessed using immunohistochemistry. Statistical analysis was performed using the Statistica 12 PL software ($p<0.05$).

Results: Analysis of EDIL3 expression showed that the average optical density of the reaction product in G1 reached 130% of the control, while the values in G2 and G3 were 153% and 158%, respectively. Regardless of the endometrial cancer grade, an increase in EDIL3 level was observed compared to the control.

Conclusion: In our study, we demonstrated overexpression of EDIL3 protein in endometrial cancer. Differences in expression between degrees of tumor differentiation suggest the potential of using changes in EDIL3 level as a new complementary diagnostic marker and target for anti-angiogenic therapy.

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1. INTRODUCTION

Extracellular matrix (ECM) proteins are the main extracellular components of the tumor microenvironment [1, 2] that affect cell growth, invasion, migration, anoikis and metastasis [3-6]. The representative of ECM proteins is Epidermal Growth Factor-like repeats and Discoidin I-Like Domains 3 (EDIL3), which was first described in the context of vascular morphogenesis [7, 8]. Physiologically, EDIL3 expression is observed only during embryonic development [8]. It is an integrin ligand that plays a key role in remodeling and developing the walls of blood vessels. The secretion of this protein has been observed in angioblasts and early endothelial cells of developing organs such as the lungs, kidneys and heart. In addition, EDIL3 affects endocytosis and programmed cell death [9, 10]. It also plays an important role in modulating the adhesion of immunocytes through interactions with leukocyte-specific integrins [11]. Changes in the

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expression profile of EDIL3 were observed in hepatocellular carcinoma [12, 13], pancreatic cancer [14], colorectal cancer [15], bladder cancer [16], breast cancer [17] and lung cancer [18]. The key aspect of cancer transformation is angiogenesis [19], during which the loss of balance between the level of pro- and anti-angiogenic factors is observed [20]. EDIL3 protein initiates angiogenesis, which indicates that tumor cells with a high level of its expression are able to stimulate endothelial cell growth and promote formation of new blood vessels [9, 21, 22].

One of the most commonly diagnosed gynecological cancers is endometrial cancer. The degree of cancer differentiation affects the probability of spread and recurrence of the disease. In the histopathological examination, a three-tier grading (G1-G3) is used based on the International Federation of Gynecology and Obstetrics (FIGO) recommendations: G1-5% or less of a solid growth pattern, G2 - between 6 and 50% of a solid growth pattern, G3 - more than 50% of a solid growth pattern [23].

The aim of this study was to investigate changes in the expression profile of EDIL3 at various degrees of endometrial cancer differentiation (G1-G3) based on immunohistochemistry. It will allow us to assess the possibility of using EDIL3 as a supplementary molecular marker in the diagnostics and searching for endometrial cancer therapy.

2. MATERIAL AND METHODS

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB/237/16). The analysis of changes in the expression profile of EDIL3 protein was performed in 45 patients who underwent hysterectomy due to diagnosed endometrial cancer in various grades of its differentiation (G1 and G2, 15 patients; G3, 13 patients). The control group (C) consisted of 15 patients without neoplastic changes during routine gynecological examinations. The analyzed groups were characterized by the following values of age, height, weight, BMI, presented as mean ± standard deviation: C = 54.57 ± 10.81 years, 1.62 m ± 0.07 m, 73.79kg ± 11.95kg, 28.21 ± 8.20-overweight; G1 = 66.63 ± 7.05 years, 1.61 m ± 0.04 m, 74.38kg ± 11.95kg, 28.77 ± 5.73 - overweight; G2 = 67.4 ± 11.89 years, 1.57 m ± 0.05 m, 84.77kg ± 25.63kg, 34.63 ± 11.63 - class I obesity; G3 = 63.38 ± 9.52 years, 1.58 m ± 0.05 m, 83.13 kg ± 14.92 kg, 33.58 ± 6.31 - class I obesity. The study did not qualify patients undergoing hormone replacement therapy in the period of 24 months prior to surgery, with BMI > 40 kg/m^2, diagnosed with endometriosis, adenomyosis, non-endometrioid endometrial cancer, coexisting cervical cancer.

To determine the expression of EDIL3 protein, paraffin blocks provided by the Laboratory of Pathomorphology of Beskid Center of Oncology in Bielsko-Biała were used. Rabbit polyclonal anti-EDIL3 antibody (Novus Biological, USA) was used to perform immunohistochemical staining of the prepared slides. The sections were incubated in citrate buffer (pH 6, 30 min at 95°C) in water bath for antigen retrieval. Then they were treated with 3% (v/v) H_2O_2 in water for 10 min to block endogenous peroxidase activity. Non-specific binding was blocked with 1% BSA solution in PBS (30 min at room temperature). Next, the slides were incubated with rabbit polyclonal anti-EDIL3 antibody (3.28 µg/ml) in a humidified chamber (20h at 4°C). Then the avidin-biotin complex (ABC) method was performed according to the manufacturer's protocol (Vectastain Elite ABC Kit; Vector Laboratories, USA). Diaminobenzidine (DAB) was used to visualize the bound antibodies. The slides were stained with Gill's hematoxylin, dehydrated, and cover-slipped. The negative control was performed by replacing the primary antibody with rabbit IgG. Photographic documentation was prepared using an Eclipse E200 light microscope with DS-Fi1 digital camera (Nikon). In order to assess the immunohistochemical reaction, a total of 15 photos were taken for each paraffin block (200x magnification). Using the NIS-AR (Nikon) program, the optical density of the reaction product was evaluated in fields where a positive immunohistochemical reaction occurred.

Statistical analysis was carried out using the Statistica 12 PL software (StatSoft, USA). First, the normality of the analyzed data was evaluated using the Kolmogorov-Smirnov test. Then one-way ANOVA and Tukey's post-hoc tests were performed ($p<0.05$). Correlations between protein level in every cancer grade and weight and BMI were determined by Pearson correlation coefficient. The results of changes in the expression of the analyzed protein are presented as mean ± standard deviation.

3. RESULTS

In the control group, EDIL3 expression was observed only in glandular cells, whereas in the study group it was present only in cancer cells (Table **1**, Fig. **1**). The arrows show the places where the expression of analyzed protein was observed. Expression of EDIL3 in glandular cells indicates that they are the source of the studied protein, and thus are involved in angiogenesis in the endometrium. In turn, EDIL3 expression in cancer cells is also associated with intense angiogenesis within the tumor as well as reduction of apoptosis and induction of cell migration in which EDIL 3 is involved. The average optical density of the immunohistochemical reaction product in G1 endometrial cancer reached 130% of the control, in G2 it was 153% of the value noted in the control, while the values in G2 and G3 were 153% and 158% respectively. The observed values indicate an increase in EDIL3 level along with the increase in cancer grade. It can also be concluded that the largest difference in its expression occurs between the G1 and G3 endometrial cancer (Table **1**). Statistically significant correlation was found only between EDIL3 expression in patients with G3 endometrial cancer and weight ($r = -0.800350$) and BMI ($r = -0.810750$). The value of the correlation coefficient indicates a strong relationship between the analyzed parameters.

4. DISCUSSION

The search for new markers to identify carcinogenesis at the early stage of neoplastic transformation plays a key role in modern diagnostics and significantly increases the chances of curing and achieving remission in a patient. The huge possibilities of detection by marker systems result from the development of molecular biology and proteomics methods [24]. The introduction of a liquid biopsy for wider diagnostics is linked with the chance of detecting cancers associated

Table 1. Optical density of the reaction product for EDIL3 in different grades of endometrial cancer and control.

Localization	Control	G1	G2	G3
Glandular cells	110.6 ± 9.3	-	-	-
Cancer cells	-	144.7 ± 12.4[a]	168.1 ± 13.5[a]	173.7 ± 15.8[a]

a - statistically significant changes at $p<0.05$ (G1 *vs.* C; G2 *vs.* C; G3 *vs.* C; G3 *vs.* G2; G3 *vs.* G1).

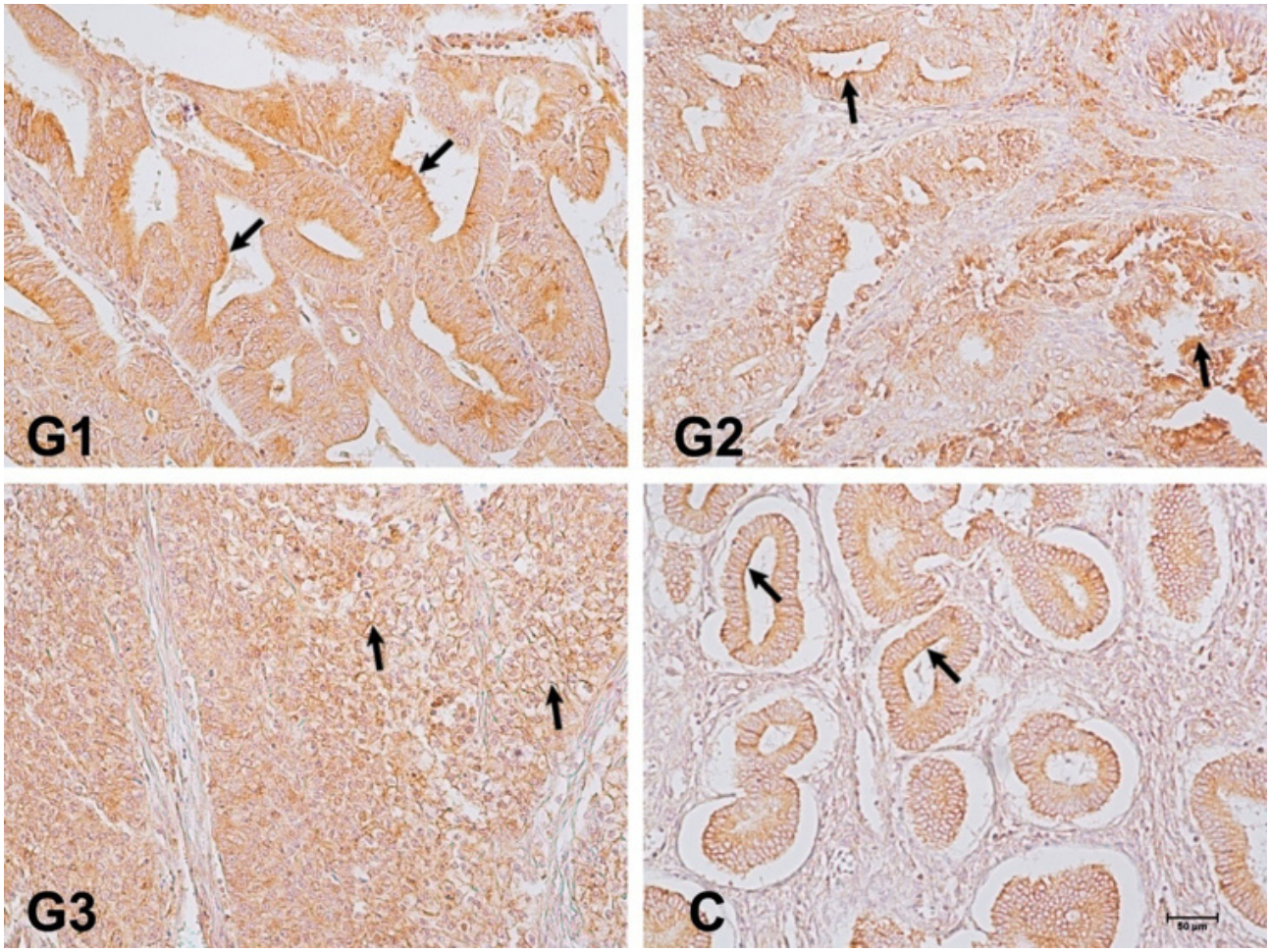

Fig. (1). Immunohistochemical localization of EDIL3 in different grades of endometrial cancer (200x magnification). C, control; G, grade of endometrial cancer.

with viral infections in an early stage of tumor development. However, the role of proteomic studies in cancer diagnostics should not be diminished. They have been conducted and analyzed for a long time, thus allowing for a more comprehensive understanding of changes at the molecular level [25, 26]. In addition, it is also important to search for new therapeutic goals, including those based on anti-angiogenic therapy [27].

In this study, we evaluated changes in EDIL3 protein level at three degrees of endometrial cancer differentiation and determined their utility as a complementary molecular marker. It is extremely important to find a marker whose changes in expression indicate the progression of carcinogenesis, but at the same time allow to reliably differentiate between healthy and pathological tissues. Studies at the transcriptome level based on the oligonucleotide microarray technique (HG-U133A; Affymetrix, Inc., Santa Clara, CA, USA) have shown that *EDIL3* differentiates G1 endometrial cancer from control (normal endometrium) [28]. We observed its overexpression (FC = + 1.791), which coincides with the optical density of the reaction product for EDIL3 in G1 endometrial cancer tissue samples observed in this work. The microarray analysis also showed that changes in the transcriptional activity of *EDIL3* in G2 and G3 endometrial cancer are statistically insignificant when compared to the control, and therefore it is not a differentiating gene for these grades [28].

Modulation of *EDIL3* transcriptional activity involves an epigenetic mechanism of the sequence-specific regulation of gene expression by miRNAs. Xia *et al.* in their studies on hepatocellular carcinoma demonstrated miR-137 participation in the regulation of EDIL3 [29]. If such mechanism is

also present in endometrial cancer, this would indicate that, firstly, miRNAs play a role not only in the silencing of gene expression [30], but also in enhancing their transcriptional activity. Secondly, perhaps in a certain tissue the interaction strength between mRNA and miRNA is too weak and does not translate into lower expression of the target transcript [31]. It is also possible to overlap these two hypotheses. Cobb *et al.* emphasize the role of environmental factors in cancer development, thus determining the chances of effective therapy. They indicate the relationship between the incidence of obesity and the development of endometrial cancer, as well as hyperglycemia with a non-physiological expression profile of proangiogenic genes [32]. In our study, statistically significant correlation was found only between EDIL3 level and weight and BMI in G3 endometrial cancer. We demonstrated that with the increase in these parameters, the expression of the analyzed protein is silenced. Our observations are inconsistent with the results presented by Cobb et al, who found an increase in EDIL3 expression as the BMI increased. A potential cause of these discrepancies may be the fact that the analysis was carried out on the mouse model [32]. Observation of a significant correlation only in patients with G3 endometrial cancer may be due to poor tumor differentiation. Thus, the disturbance of the body homeostasis is the highest compared to the earlier grades, which is accompanied by a change in the concentrations of cytokines, whose activity promotes metabolic changes [33]. Another potential reason for the observed correlation and its direction may be the size of the study group and the life environment of patients.

EDIL3 is described as an extracellular matrix protein, significantly affecting the severity of tumor angiogenesis [9, 21, 22, 34]. Xia *et al.* indicate that EDIL3 activates the TGF-β and ERK pathways. They emphasize the role of the analyzed protein in angiogenesis, metastasis and recurrences of hepatocellular carcinoma. Their analysis was performed at the transcriptome and proteome levels [29]. Our work seems to be a valuable study of EDIL3 expression at the proteome level, which allows us to observe changes in its expression in the entire DNA-RNA-protein genetic information flow. Studies of Zhang *et al.* confirm the effect of EDIL3 on the TGFβ signaling pathway and epithelial-mesenchymal transition (EMT) in epithelial cells. The reduced expression of EDIL3 was accompanied by a decreased level of α-actin and vimentin, a decrease in SMAD2 and SMAD3 phosphorylation and activation of extracellular signal-regulated kinase (ERK). They indicate that in addition to the possibility of using EDIL3 as a diagnostic marker, it is an interesting and promising therapeutic target for posterior capsule opacification [35]. The possibility of making EDIL3 a therapeutic target in breast cancer is underlined [17], suggesting an extension of its potential therapeutic use to other gynecological malignancies, such as endometrial cancer. In ovarian endometriosis, an increase in EDIL3 expression was also observed (FC = 3.44, p = 0.017292) [36]. Our current work combined with the previous one [28] and observations made by other research teams is a valuable complement and arrangement of knowledge about changes in the expression profile of EDIL3 in cancer. Lee *et al.* also emphasize the role of EDIL3 as an independent prognostic factor of survival and a condition for its development, independent of anchoring the tumor. They indicated that as the expression of this protein increases, the survival time is shortened. These researchers also described the effect of EDIL3 on the balance between pro- and anti-apoptotic proteins. Their observations indicate that as the level of EDIL3 increases, the expression of Bcl-2 and Bcl-x also increases, while the Bax level changes slightly [17]. The detection of EDIL3 in normal pancreatic tissues is an intriguing observation. Jiang *et al.* associate this unexpected observation with the influence of the tumor microenvironment on normal tissues [37]. It is suggested that EDIL3 interacts and modulates the tumor microenvironment *via* angiogenic pathways [32].

An important aspect of the research is to find the best method for quantifying the changes in the expression of the potential marker. Lee *et al.* in their study on the level of EDIL3 in breast cancer used the immunohistochemical staining method and mass spectrometry (MS), indicating the mutual complementation of the obtained results [17]. Beckham *et al.* also used the MS method and obtained strong premises for accurate assessment of the expression pattern of EDIL3 in bladder cancer [16]. Other researchers also emphasize the complementation of immunohistochemical staining and MS in the assessment of changes in concentrations of the analyzed factors. Thus, they do not deny any of them for the benefit of the other, although it seems that MS goes beyond classical histology [38-40]. Lee *et al.*, Kaweewong *et al.* and Stamer *et al.* indicate the possibility of extraction and solubilization of proteins, including EDIL3, from tissues and then conducting sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine the concentration profile of the analyzed proteins [17, 41, 42]. All of these observations show the diversity of methods for assessing protein expression and their complementation, which gives the possibility of a holistic view of the importance of a given protein in a specific pathological condition.

Based on the immunohistochemical analysis performed in this study, it can be concluded that with the increase of endometrial cancer grade, EDIL3 level increases and is always higher than in the control. The highest differences in concentrations of EDIL3 occur between G1 and G2, as well as G1 and G3 (Table **1**). The observed upward trend in the expression of the discussed protein along with the increase of endometrial cancer grade suggests the ease of interpretation of the expression results, indicating the value of introducing EDIL3 as a new molecular marker in the diagnosis of endometrial cancer. In addition, the observed relatively large differences in the protein level between individual grades suggest the possibility of complementing the histopathological examination of the endometrial cancer grade with the assessment of molecular differentiation by determining the level of EDIL3. Jeong *et al.* observed the same trend of EDIL3 expression at mRNA and protein levels in lung adenocarcinoma and squamous cell lung cancer. They noted a correlation between EDIL3 and the mesenchymal phenotype of tumor cells by assessing the EMT markers (E-cadherin, β-catenin, vimentin and CD31). Based on the multivariate analysis, they concluded that EDIL3 acts as an independent predictor of overall survival in lung adenocarcinoma. Thus, the analyzed protein is closely related to angiogenesis, the development of tumor mass in lung adenocarcinoma and mesenchymal phenotype [18].

An extremely important aspect of any diagnostics, in addition to its utilitarian value, is to minimize the patient's physical and mental suffering, use of burdensome procedures for the patient, which include complicated research and the long-awaited outcome. Procedures carried out in our work eliminated the appearance of additional negative experiences in patients treated oncologically. The study used tissue samples from which histopathological examination was performed, allowing to determine the degree of cancer differentiation. Also, the methodology of the study and the time expected for the result is relatively short and does not interfere with other medical procedures. The presented arguments indicate the value of molecular research, including the determination of EDIL3 expression and the possibility to incorporate them in modern oncological treatment. Therefore, it is not possible to analyze only one signaling pathway in an isolated system due to the interactions between the pathways and the molecules involved [43]. For example, Yin *et al.* indicate a key contribution of the HAND2 gene and protein expression in the induction and development of endometrial cancer of emphasizing the possibility of using it as a molecular marker in this tumor [44]. This is also confirmed in our previous works, in which we presented the potential diagnostic value of endoglin and neuropilins assessment among patients with endometrial cancer [45, 46]. Thus, the connections between particular factors and the value of analyzing changes in the expression of defined groups of genes and proteins become more visible.

CONCLUSION

In conclusion, the analysis of changes in the expression of EDIL3 protein suggests its potential use as a complementary molecular marker in the diagnosis and determination of the endometrial cancer grade. A thorough analysis of the literature data with the results obtained in the previous [26] and this work indicates the complexity of neoplastic transformation and the key role of the microenvironment. The presented study is important as there are relatively few works analyzing the expression of EDIL3 in endometrial cancer.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approval of the Bioethical Committee of the Medical University of Silesia, No. KNW/0022/KB/237/16 has been obtained for this study.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All human procedures were followed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

CONSENT FOR PUBLICATION

Informed consent was obtained from all of the patients recruited.

AVAILABILITY OF DATA AND MATERIALS

All data was included in the manuscript.

FUNDING

None.

CONFLICT OF INTEREST

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

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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.

REFERENCES

[1] Hynes, R.O. The extracellular matrix, not just pretty fibrils. *Science*, **2009**, *326*(5957), 1216-1219.

[2] Ma, M.Z.; Zhuang, C.; Yang, X.M.; Zhang, Z.Z.; Ma, H.; Zhang, W.M.; You, H.; Qin, W.; Gu, J.; Yang, S.; Cao, H.; Zhang, Z.G. CTHRC acts as a prognostic factor and promotes invasiveness of gastrointestinal stromal tumors by activating Wnt/PCP-Rho signaling. *Neoplasia,* **2014**, *16*(3), 265-278.

[3] Ye, Q.H.; Qin, L.X.; Forgues, M.; He, P.; Kim, J.W.; Peng, A.C.; Simon, R.; Li, Y.; Robles, A.I.; Chen, Y.; Ma, Z.C.; Wu, Z.Q.; Ye, S.L.; Liu, Y.K.; Tang, Z.Y.; Wang, X.W. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat. Med*., **2003**, *9*(4), 416-423.

[4] Bergamini, C.; Sgarra, C.; Trerotoli, P.; Lupo, L.; Azzariti, A.; Antonaci, S.; Giannelli, G. Laminin-5 stimulates hepatocellular carcinoma growth through a different function of alpha6beta4 and alpha3beta1 integrins. *Hepatology,* **2007**, *46*(6), 1801-1809.

[5] Li, H.; Ge, C.; Zhao, F.; Yan, M.; Hu, C.; Jia, D.; Tian, H.; Zhu, M.; Chen, T.; Jiang, G.; Xie, H.; Cui, Y.; Gu, J.; Tu, H.; He, X.; Yao, M.; Liu, Y.; Li, J. Hypoxia-inducible factor 1 alpha-activated angiopoietin-like protein 4 contributes to tumor metastasis *via* vascular cell adhesion molecule-1/integrin β1 signaling in human hepatocellular carcinoma. *Hepatology*, **2011**, *54*(3), 910-919.

[6] Muir, A.; Danai, L.V.; Vander Heiden, M.G. Microenvironmental regulation of cancer cell metabolism, implications for experimental design and translational studies. *Dis. Model Mech.,* **2018**, *11*(8), pii, dmm035758.

[7] Camussi, G.; Deregibus, M.C.; Bruno, S.; Cantaluppi, V.; Biancone, L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int.,* **2010**, *78*(9), 838-848.

[8] Hidai. C.; Zupancic, T.; Penta, K.; Mikhail, A.; Kawana, M.; Quertermous, E.E.; Aoka, Y.; Fukagawa, M.; Matsui, Y.; Platika, D.; Auerbach, R.; Hogan, B.L.; Snodgrass, R.; Quertermous, T. Cloning and characterization of developmental endothelial locus-1, an embryonic endothelial cell protein that binds the alphavbeta3 integrin receptor. *Genes Dev.,* **1998**, *12*(1), 21-33.

[9] Zhong, J.; Eliceiri, B.; Stupack, D.; Penta, K.; Sakamoto, G.; Quertermous, T.; Coleman, M.; Boudreau, N.; Varner, J.A. Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del-1. *J. Clin. Invest.,* **2003**, *112*(1), 30-41.

[10] Ho, H.K.; Jang, J.J.; Kaji, S.; Spektor, G.; Fong, A.; Yang, P.; Hu, B.S.; Schatzman, R.; Quertermous, T.; Cooke, J.P. Developmental endothelial locus-1 (Del-1), a novel angiogenic protein, its role in ischemia. *Circulation,* **2004**, *109*(10), 1314-1319.

[11] Choi, E.Y.; Chavakis, E.; Czabanka, M.A.; Langer, H.F.; Fraemohs, L.; Economopoulou, M.; Kundu, R.K.; Orlandi, A.; Zheng, Y.Y.; Prieto, D.A.; Ballantyne, C.M.; Constant, S.L., Aird, W.C.; Papayannopoulou, T.; Gahmberg, C.G.; Udey, M.C., Vajkoczy, P.; Quertermous, T.; Dimmeler, S.; Weber, C.; Chavakis, T. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. *Science,* **2008**, *322*(5904), 1101-1104.

[12] Sun, J.C.; Liang, X.T.; Pan, K.; Wang, H.; Zhao, J.J., Li, J.J., Ma, H.Q., Chen, Y.B., Xia, J.C. High expression level of EDIL3 in HCC predicts poor prognosis of HCC patients. *World J. Gastroenterol.,* **2010**, *16*(36), 4611-4615.

[13] Luo, J.H.; Ren, B.; Keryanov, S.; Tseng, G.C.; Rao, U.N.; Monga, S.P.; Strom, S.; Demetris, A.J.; Nalesnik, M.; Yu, Y.P. Ranganathan, S.; Michalopoulos, G.K. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. *Hepatology,* **2006**, *44*(4), 1012-1024.

[14] Damhofer, H.; Medema, J.P.; Veenstra, V.L.; Badea, L.; Popescu, I.; Roelink, H.; Bijlsma, M.F. Assessment of the stromal contribution to Sonic Hedgehog-dependent pancreatic adenocarcinoma. *Mol. Oncol.,* **2013**, *7*(6), 1031-1042.

[15] Watanabe, T.; Kobunai, T.; Yamamoto, Y.; Ikeuchi, H.; Matsuda, K.; Ishihara, S.; Nozawa, K.; Iinuma, H.; Kanazawa, T.; Tanaka, T.; Yokoyama, T.; Konishi, T.; Eshima, K.; Ajioka, Y.; Hibi, T.; Watanabe, M.; Muto, T.; Nagawa, H. Predicting ulcerative colitis-associated colorectal cancer using reverse-transcription polymerase chain reaction analysis. *Clin. Colorectal Cancer*, **2011**, *10*(2), 134-141.

[16] Beckham, C.J.; Olsen, J.; Yin, P.N.; Wu, C.H.; Ting, H.J.; Hagen, F.K.; Scosyrev, E.; Messing, E.M.; Lee, Y.F. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression. *J. Urol.*, **2014**, *192*(2), 583-592.

[17] Lee, J.E.; Moon, P.G.; Cho, Y.E.; Kim, Y.B.; Kim, I.S.; Park, H.; Baek, M.C. Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion. *J. Proteomics*, **2016**, *131*, 17-28.

[18] Jeong, D.; Ban, S.; Oh, S.; Jin Lee, S.; Yong Park, S.; Koh, Y.W. Prognostic significance of EDIL3 expression and correlation with mesenchymal phenotype and microvessel density in lung adenocarcinoma. *Sci. Rep.,* **2017**, *7*(1), 8649.

[19] Yang, Z.F.; Poon, R.T. Vascular changes in hepatocellular carcinoma. *Anat. Rec. (Hoboken)*, **2008**, *291*(6), 721-734.

[20] Pang, R.; Poon, R.T. Angiogenesis and antiangiogenic therapy in hepatocellular carcinoma. *Cancer Lett.*, **2006**, *242*(2), 151-167.

[21] Niu, J.X.; Zhang, W.J.; Ye, L.Y.; Wu, L.Q.; Zhu, G.J.; Yang, Z.H.; Grau, G.E., Lou, J.N. The role of adhesion molecules, alpha v beta 3, alpha v beta 5 and their ligands in the tumor cell and endothelial cell adhesion. *Eur. J. Cancer Prev.*, **2007**, *16*(6), 517-527.

[22] Wang, Z.; Longaker, M.T.; Yang, G.P. Anti-apoptotic effects of the angiogenic factor Del1 on endothelial cells. *J. Surg. Res.,* **2006**, *130*, 237.

[23] Helpman, L.; Kupets, R.; Covens, A.; Saad, R.S.; Khalifa, M.A.; Ismiil, N.; Ghorab, Z.; Dubé, V.; Nofech-Mozes, S. Assessment of endometrial sampling as a predictor of final surgical pathology in endometrial cancer. *Br. J. Cancer*, **2014**, *110*(3), 609-615.

[24] Greenwald, Z.R.; El-Zein, M.; Bouten, S.; Ensha, H.; Vazquez, F.L.; Franco, E.L. Mobile screening units for the early detection of cancer, a systematic review. *Cancer Epidemiol. Biomarkers Prev.*, **2017**, *26*(12), 1679-1694.

[25] Heitzer, E.; Perakis, S.; Geigl, J.B.; Speicher, M.R. The potential of liquid biopsies for the early detection of cancer. *NPJ Precis. Oncol.,* **2017**, *1*(1), 36.

[26] Wulfkuhle, J.D.; Liotta, L.A.; Petricoin, E.F. Proteomic applications for the early detection of cancer. *Nat. Rev. Cancer*, **2003**, 3(4), 267-275.

[27] Coultas, L.; Chawengsaksophak, K.; Rossant, J. Endothelial cells and VEGF in vascular development. *Nature,* **2005**, *438*(7070), 937-945.

[28] Opławski, M.; Michalski, M.; Witek, A.; Michalski, B.; Zmarzły, N.; Jęda-Golonka, A.; Styblińska, M.; Gola, J.; Kasprzyk-Żyszczyńska, M.; Mazurek, U.; Plewka, A. Identification of a gene expression profile associated with the regulation of angiogenesis in endometrial cancer. *Mol. Med. Rep.,* **2017**, *16*(3), 2547-2555

[29] Xia, H.; Jianxiang, C.; Shi, M.; Gao, H.; Karthik, S.; Pratap, S.V.; Ooi, L.L.; Hui, K.M. EDIL3 is a novel regulator of epithelial mesenchymal transition controlling early recurrence of hepatocellular carcinoma. *J. Hepatol.*, **2015**, *63*(4), 863-873.

[30] Grenda, A.; Budzyński, M.; Filip, A. Biogeneza cząsteczek mikrona oraz ich znaczenie w powstawaniu i przebiegu wybranych zaburzeń hematologicznych. *Postępy. Hig. Med. Dosw. (online)*, **2013**, *67*, 174-185.

[31] Betel, D.; Wilson, M.; Gabow, A.; Marks, D.S.; Sander, C. The microRNA.org resource, targets and expression. *Nucleic Acids Res.*, **2008**, *36*(Database issue), D149-D153.

[32] Cobb, L.P.; Siamakpour-Reihani, S.; Zhang, D.; Owzar, K.; Berchuck, A.; Bae-Jump, V.L.; Secord, A.A. Obesity is associated with altered angiogenic gene expression in endometrioid endometrial cancer. *Gynecol. Oncol.*, **2018**, *149*(1), 177-178

[33] Szymkowiak J. Otyłość jako rodzaj przewlekłego zapalenia. *Wszechświat,* **2011**, *112*(10), 264-272.

[34] Lopes-Bastos, B.M.; Jiang, W.G.; Cai, J. Tumour-endothelial cell communications, important and indispensable mediators of tumour angiogenesis. *Anticancer Res.,* **2016**, *36*(3), 1119-1126.

[35] Zhang, R.; Wei; Y.H., Zhao, C.Y.; Song, H.Y.; Shen, N.; Cui, X.; Gao, X.; Qi, Z.T.; Zhong, M.; Shen, W. EDIL3 depletion suppress epithelial-mesenchymal transition of lens epithelial cells *via* transforming growth factor β pathway. *Int. J. Ophthalmol.*, **2018**, *11*(1), 18-24.

[36] Vouk, K.; Smuc, T.; Guggenberger. C.; Ribič-Pucelj, M.; Sinkovec, J.; Husen, B.; Thole, H.; Houba, P.; Thaete, C.; Adamski, J.; Rižner, T.L. Novel estrogen-related genes and potential biomarkers of ovarian endometriosis identified by differential expression analysis. *J. Steroid Biochem. Mol. Biol.*, **2011**, *125*(3-5), 231-224.

[37] Jiang, S.H.; Wang, Y.; Yang, J.Y.; Li, J.; Feng, M.X.; Wang, Y.H.; Yang, X.M.; He, P.; Tian, G.A.; Zhang, X.X.; Li, Q.; Cao, X.Y.; Huo, Y.M.; Yang, M.W.; Fu, X.L.; Li, J.; Liu, D.J.; Dai, M.; Wen, S.Y.; Gu, J.R.; Hong, J.; Hua, R.; Zhang, Z.G.; Sun. Y.W. Overexpressed EDIL3 predicts poor prognosis and promotes anchorage-independent tumor growth in human pancreatic cancer. *Oncotarget,* **2016**, *7*(4), 4226-4240.

[38] Jones, G.N.; Rooney, C.; Griffin, N.; Roudier, M.; Young, L.A.; Garcia-Trinidad, A.; Hughes, G.D.; Whiteaker, J.R.; Wilson, Z.; Odedra, R.; Zhao, L.; Ivey, R.G.; Howat, W.J.; Harrington, E.A.; Barrett, J.C.; Ramos-Montoya, A.; Lau, A.; Paulovich, A.G.; Cadogan, E.B.; Pierce, A.J. pRAD50, a novel and clinically applicable pharmacodynamic biomarker of both ATM and ATR inhibition identified using mass spectrometry and immunohistochemistry. *Br. J. Cancer*, **2018**, *119*(10), 1233-1243.

[39] Seuma, J.; Bunch, J.; Cox, A.; McLeod. C.; Bell. J.; Murray, C. Combination of immunohistochemistry and laser ablation ICP mass spectrometry for imaging of cancer biomarkers. *Proteomics,* **2008**, *8*(18), 3775-3784.

[40] Schwamborn, K.; Caprioli, R.M. Molecular imaging by mass spectrometry-looking beyond classical histology. *Nat. Rev. Cancer*, **2010**, *10*(9), 639-646.

[41] Kaweewong, K.; Garnjanagoonchorn, W.; Jirapakkul, W.; Roytrakul, S. Solubilization and identification of hen eggshell membrane proteins during different times of chicken embryo development using the proteomic approach. *Protein J.*, **2013**, *32*(4), 297-308.

[42] Stamer, W.D.; Hoffman, E.A.; Luthe,r J.M.; Hache,y D.L.; Schey, K.L. Protein profile of exosomes from trabecular meshwork cells. *J. Proteomics*, **2011**, *74*(6), 796-804.

[43] Netea, M.G.; Balkwill, F.; Chonchol, M.; Cominelli, F.; Donath, M.Y.; Giamarellos-Bourboulis, E.J.; Golenbock, D.; Gresnigt, M.S.; Heneka, M.T.; Hoffman, H.M.; Hotchkiss, R.; Joosten, L.A.B.; Kastner, D.L.; Korte, M.; Latz, E.; Libby, P.; Mandrup-Poulsen, T.; Mantovani, A.; Mills, K.H.G.; Nowak, K.L.; O'Neill, L.A., Pickkers, P.; van der Poll, T.; Ridker, P.M.; Schalkwijk, J.; Schwartz, D.A.; Siegmund, B.; Steer, C.J.; Tilg, H.; van der Meer, J.W.M.; van de Veerdonk, F.L.; Dinarello, C.A. A guiding map for inflammation. *Nat. Immunol.*, **2017**, *18*(8), 826-831.

[44] Yin, X.H.; Shi, M.; Wu, H.; Zhou, L.; Xu, B. Role of HAND2 gene and protein expression in endometrial carcinoma. *Eur. J. Gynaecol. Oncol.*, **2017**, *38*(1), 95-101.

[45] Opławski, M.; Dziobek, K.; Adwent, I.; Dąbruś, D.; Grabarek, B.; Zmarzły, N.; Plewka, A.; Boroń, D. Expression profile of endoglin in different grades of endometrial cancer. *Curr. Pharm. Biotechnol*., **2018**, *19*(12), 990-995.

[46] Opławski, M.; Dziobek, K.; Grabarek, B.; Zmarzły, N.; Dąbruś, D.; Januszyk, P.; Brus, R.; Tomala, B.; Boroń, D. Expression of NRP-1 and NRP-2 in Endometrial Cancer. *Curr. Pharm. Biotechnol*., **2018**, *20*, 1-7.