Rev Med Chile 2016; 144: 1577-1583

GRP78 levels, regional fat distribution and endometrial cancer

RĂZVAN CIORTEA, ANDREI M. MALUTAN, LENUTA M. ANGHELUTA, CARMEN E. BUCURI, MARIA P. RADA, DAN MIHU

Department of Obstetrics and Gynecology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Financial support for this work: This paper was published under the frame of The European Social Fund, Human Resources Development Operational Programme 2007-2013, project no.7135/5/30.04.2014 POSDRU.

The authors declare no conflict of interest.

Recibido el 21 de mayo de 2016, aceptado el 10 octubre de 2016.

Coresponding author:
Ciortea Razvan
Bd 21st Decembrie no. 55-57.
Cluj-Napoca, Romania.
Tel. 0040748030897
r_ciortea@yahoo.com

ABSTRACT

Background: *The association of obesity with endometrial cancer is supported by the presence of endoplasmic reticulum (ER) stress in the adipocyte. Glucose-regulated protein 78 (GRP78) is a marker for ER stress. This protein is a central regulator of ER stress due to its major anti-apoptotic role. It plays an important role in tumor development, progression and chemoresistance.* ***Aim****: To look for an association between android and gynoid obesity, plasma GRP78 levels and endometrial cancer.* ***Material and methods****: Forty four patients with endometrial cancer aged 72 ± 6 years and 44 healthy women aged 55 ± 9 years were studied. Android and gynoid fat distribution were determined by dual X-ray absorptiometry and plasma GRP78 levels were measured.* ***Results****: GRP78 plasma levels were significantly higher in patients with endometrial cancer as compared to the control group. Android fat distribution had a positive correlation with plasma GRP78 levels (p<0.01). Gynoid fat had a negative correlation with plasma GRP78 levels (p<0.01).* ***Conclusions:*** *GRP78 levels are associated with the distribution of adipose tissue and are higher in patients with endometrial cancer.*

(Rev Med Chile 2016; 144: 1577-1583)

Key words: *Adipose Tissue; Endometrial Neoplasms; Obesity; Endoplasmic Reticulum Stress.*

Niveles de GRP78, distribución regional del tejido adiposo y cáncer endometrial

Antecedentes*: La asociación de obesidad con cáncer endometrial puede depender de la presencia de estrés del retículo endoplásmico (RE) en el adipocito. La proteína 78 regulada por glucosa (GRP78) es un marcador de estrés del RE. Esta proteína regula el estrés de RE gracias a su rol antiaopoptótico. Ella juega un rol en el desarrollo, progresión y quimio-resistencia de tumores.* ***Objetivo****: Buscar una asociación entre obesidad androide o ginoide, niveles plasmáticos de GRP78 y cáncer endometrial.* ***Material y métodos****: Se estudiaron 44 mujeres con cáncer endometrial de 72 ± 6 años and 44 mujeres sanas de 55 ± 9 años. La distribución androide o ginoide de la grasa fue determinada por densitometría radiológica de doble fotón (DEXA) y se midieron los niveles plasmáticos de GRP78.* ***Resultados:*** *Los niveles de GRP78 fueron significativamente más altos en mujeres con cáncer endometrial. Se observó una correlación positiva entre la distribución de grasa androide y los niveles de GRP78 (p< 0.01). Se observó una correlación negativa entre distribución de grasa ginoide y niveles de GRP78.* ***Conclusiones****: Los niveles de GRP78 se correlacionan con la distribución del tejido adiposo y son mayores en mujeres con cáncer endometrial.*

Starting from the idea that in modern society the prevalence of obesity is continuously increasing, that adipose tissue is directly correlated with several disorders, and that there are a series of paraclinical investigations specific to adipose tissue, adipose tissue dysfunction is currently considered an individual pathological condition.

In economically developed countries, endometrial cancer (EC) is associated with obesity in a proportion of 40%[1]. The hypothesis of the alteration of the estrogen-progesterone balance is used to support the relationship between obesity, endogenous steroid hormones and the risk of EC, but it cannot explain the effects on the entire population, given that not all obese women develop endometrial abnormalities. The identification of biological markers that indicate an increased risk for the development or recurrence of EC in obese women might be useful for decreasing EC mortality and morbidity.

The strong association between obesity and endometrial cancer can be due to the development of stress endoplasmic reticulum (ER) in adipocytes. Stress ER is an adaptive reaction, which normally occurs in any cell that is required to process more molecules than usual per time unit. In this situation, there is a risk that part of the incompletely processed molecules (post-translational molecular folding, twisting, packing) may generate an adaptive reaction in ER that consists in the occurrence of molecular conformational defects, which might prevent traffic through this structure[2].

Glucose-regulated protein 78 (GRP78) is in the endoplasmic reticulum and it is an important regulator of the unfolded protein response[3]. GRP78 supports cell survival, having anti-apoptotic properties[4]. GRP78 levels in visceral adipocytes are correlated with the stage of EC, but also with the survival of these patients, and might be clinically useful as a predictor for endometrial cancer[5].

Material and Methods

The study is a case-control analysis that includes 2 groups of patients: group I : 44 patients diagnosed with endometrial cancer. Group II: 44 patients without gynecological pathology or inflammatory disorders.

The diagnosis of endometrial cancer was made after histopathological examination that analyzed the tissue material obtained following endometrial biopsy. All patients enrolled in the study had endometrial adenocarcinoma, endometrioid type, grade I or II.

The anthropometric measurements performed were: body mass index (BMI), calculated by the formula BMI = weight (kg)/[height (m)]^2, and abdominal circumference (AC), measured in orthostatism, at umbilical level. The height of the subjects was accurately measured (error less than 1 mm), by the stretching procedure for height measurement of the Society for the Development of Kinanthropometry, using an anthropometer fixed to the wall (222 model; Seca GMBH, Hamburg, Germany). Body mass was determined with an accepted error of 20 g using a calibrated electronic scale (FW-150K model; A&D Mercury, Thebarton, Australia).

These patients underwent dual-energy X-ray absorptiometry (DXA) examination with a GE Prodigy Lunar device, which measured the android and gynoid adipose tissue content. The "android" and "gynoid" regions were defined using the software provided by the producer. The android region has a lower limit at the upper limit of the pelvis and an upper limit at the junction of the lower 1/5 with the upper 4/5 of the pelvis-to-chin distance. Lateral limits are represented by the internal limits of the hands. The gynoid region has an upper limit at the upper part of the greater trochanters and a lower limit defined at the distance equal to twice the height of the android region. Lateral limits are represented by the external limits of the legs.

From each subject included in the study, 6 ml fasting blood were taken by venous puncture and collected in test tubes without anticoagulant for the determination of plasma GRP78 levels. The serum obtained by centrifugation was divided and stored in 600 µl freezing tubes at a temperature of –60^0 C until the tests were performed, in order to avoid repeated freezing-thawing cycles. The serum GRP78 concentration was determined by the sandwich ELISA technique using the Human GRP78 Immunoassay MBS031039 kit, R&D Systems, USA. The detection rate of this kit is 0,1µg/ml. No significant cross reactivity or interference between human GRP78 and analogues were found.

Case-control studies imply an appropriately pairing of subjects. Age was an independent factor

associated with GRP78 levels, so it did not influenced pairing of subjects included in this study.

The informed consent of all patients was obtained. The study was conducted under the tenets of Helsinki declaration. The Ethical Committee of the University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, approved the study.

All parameters were included in the study database. Normal distribution was tested with the Kolmogorov-Smirnov test. Normally distributed variables are presented as mean ± standard deviation; non-normally distributed as median (interquartile range). For comparison of two means the t-test or Mann-Whitney test was used. For relationship analysis between two variables, Pearson's or Spearman correlation coefficient (r) was used. To control for age partial correlation and multivariate linear regression the stepwise method was used. Statistical analysis was performed using SPSS 15.0.

Results

The characteristics of the patients included in the study are described in Table 1.

Plasmatic level of GRP78 was significant higher in patients with endometrial cancer compared to the control group.

Android fat was in a statistically significant, positive linear correlation with the plasma GRP78

Table I.Characteristics of the patients included in the study

	Grup	n	Mean	p	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Age	Martor = 0	44	55.11	< 0.0001	8.47	52.54	57.69	39	70
	Cas = 1	44	71.84		5.69	70.11	73.57	59	83
	Total	88	63.48		11.05	61.14	65.82	39	83
Menarche	0	44	13.02	0.002	1.09	12.69	13.35	11	15
	1	44	12.25		1.16	11.90	12.60	10	15
	Total	88	12.64		1.19	12.39	12.89	10	15
Menopause	0	44	46.00	Ns	3.03	45.08	46.92	40	54
	1	44	51.95		3.38	50.93	52.98	40	59
	Total	88	48.98		4.38	48.05	49.90	40	59
Weight(kg)	0	44	85.61	Ns	8.35	83.08	88.15	64	100
	1	44	86.89		8.37	84.34	89.43	67	102
	Total	88	86.25		8.33	84.48	88.02	64	102
BMI	0	44	31.23	Ns	2.88	30.35	32.10	23	37
	1	44	31.89		2.93	31.00	32.78	26	38
	Total	88	31.56		2.91	30.94	32.17	23	38
AC(cm)	0	44	90.86	< 0.0001	12.75	86.99	94.74	70	118
	1	44	104.05		11.30	100.61	107.48	86	124
	Total	88	97.45		13.69	94.55	100.36	70	124
% Android fat	0	44	45.43	< 0.0001	6.19	43.55	47.31	35	57
	1	44	56.75		4.12	55.50	58.00	48	64
	Total	88	51.09		7.73	49.45	52.73	35	64
% Gynoid fat	0	44	47.93	< 0.0001	5.97	46.12	49.75	36	58
	1	44	34.18		4.34	32.86	35.50	28	45
	Total	88	41.06		8.64	39.23	42.89	28	58
GRP78 (μg/ml)	0	44	0.379	< 0.0001	0.188	0.322	0.436	0.081	0.85
	1	44	1.138		0.095	1.110	1.167	0.989	1.329
	Total	88	0.759		0.410	0.672	0.846	0.081	1.329

level (Figure 1). The correlation coefficient between android fat and the plasma GRP78 level was $R = 0.4431$, $p < 0.0001$. This coefficient suggests an influence of 44% in plasma GRP78 level variance due to android fat.

Increased gynoid fat was correlated also with low plasma GRP78 levels (Figure 2) with a correlation coefficient of 0.4792, $p < 0.0001$.

In order to establish the influence of the other variables on GRP78, the following variables were entered in regression: age, menarche, menopause, android fat, age, menopause and android fat proved to be independent factors associated with GRP78 level (Table 2).

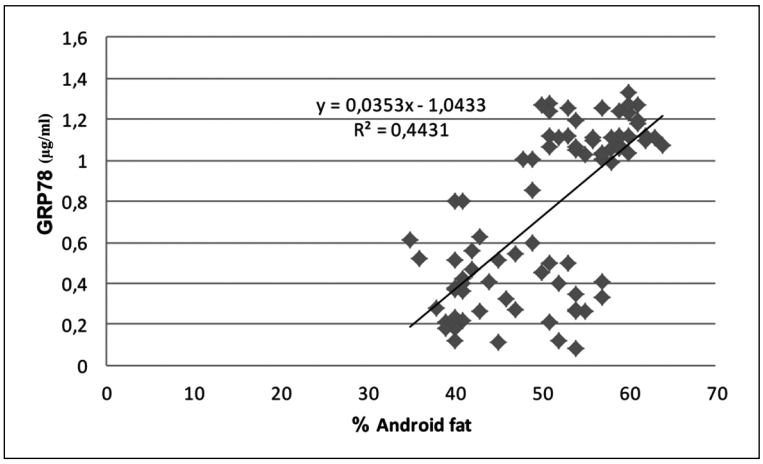

Figure 1. Correlation between GRP78 and android fat.

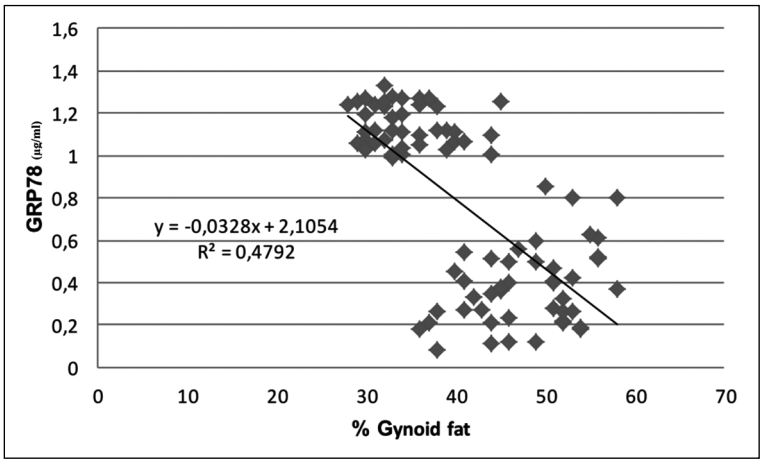

Figure 2. Correlation between GRP78 and gynoid fat.

Table 2. Multivariate linear regression analysis

Independent variables	Coefficient	Std.Error	t	P
(Constant)	-2.0395			
Age	0.01603	0.002952	5.430	<0.0001
Menarche	-0.01641	0.02288	-0.717	0.4753
% Android fat	0.01399	0.004286	3.263	0.0016
Menopause	0.0260	0.00716	3.632	0.0005

Dependent variable: GRP78.

Discussion and conclusions

Depending on the predominance of adipose tissue and on its distribution, obesity is android and gynoid. Android obesity is characterized by the predominance of adipose tissue in the central region of the body, in the abdominal wall and mesentery. Gynoid obesity is characterized by the predominance of adipose tissue in the lower part of the body, in the buttocks and thigh region. Android obesity is associated with hyperinsulinemia, decreased glucose tolerance, diabetes mellitus, increased secretion of androgens, testosterone, as well as decreased sex hormone binding globulin (SHBG) levels[6]. All these pathological conditions associated with android obesity are risk factors for endometrial cancer. This study supports the idea that android obesity is a risk factor for endometrial cancer, as there is a statistically significant difference in abdominal fat between the two groups.

Women with android obesity have an increased adrenal gland activity, with the secondary increase of ACTH and cortisol secretion, associated with hyperinsulinemia, decreased glucose tolerance, diabetes mellitus, an increase of androgen and testosterone secretion, as well as a reduction of SHBG[7].

The association of android obesity with hyperinsulinemia can be explained by three possible mechanisms[8]:

- Android obesity is more sensitive to catecholamines and less sensitive to insulin, which induces an increase of free fatty acid concentrations and consequently, hyperglycemia.
- Androgens inhibit insulin action at hepatic and peripheral level.
- Hepatic insulin extraction is inhibited by androgens and free fatty acids.

Also, there are statistically significant correlations between abdominal circumference and insulinemia, which evidences the pathogenic effect of abdominal obesity in insulin resistance and compensatory hyperinsulinemia. Thus, larger abdominal circumference, is related with higher the insulin levels. If there is a significant correlation between abdominal circumference and insulinemia, the authors cannot describe the same correlation between BMI and insulinemia, which shows that abdominal obesity has a high insulin resistance potential, unlike gluteal-femoral obesity[9].

Chronic hyperinsulinemia as a risk factor in endometrial cancer is explained at two levels[10]:

- At endometrial level, insulin stimulates tumor development by a decrease of IGFBP1 and an increase of IGF1. Insulin may directly trigger tumor transformation through endometrial receptors.
- At ovarian level, it induces hyperandrogenia, which determines anovulation, resulting in a progesterone deficit.

Insulin and insulin-like growth factor-1 (IGF-1) stimulate protein synthesis, anti-apoptotic proliferation and signaling through the induction of mitogen activated protein kinase (MAPK). GRP78 expression is a target downstream of insulin, recent evidence suggesting that GRP78 and the global protein balance of the endoplasmic reticulum can regulate the insulin sensitivity of the body and can protect cells during acute stress. Obesity and type 2 diabetes mellitus are metabolic disorders characterized by insulin resistance and hepatic steatosis. The presence of ER stress in the context of metabolic syndrome has been documented[11,12], and the presence of chaperones might play a key role in the regulation of insulin sensitivity and glucose homeostasis. The administration of chemical chaperones to obese mice reduced stress ER markers, restored glucose levels and insulin homeostasis, while weight gain and the increase of hepatic insulin sensitivity were low[13]. GRP78 was diminished in the adipose tissue of patients with gastric bypass after weight loss, which means that the relationship between obesity-related stress ER and metabolic dysfunction is present in humans[14].

Stress ER has been extensively studied because it can contribute to the development of severe diseases such as neurodegenerative diseases, type 2 diabetes mellitus or different forms of cancer[15]. Recent studies have demonstrated that endoplasmic reticulum stress leads to the alteration of lipid metabolism and hepatic steatosis, because certain components of the signaling system through the unfolded protein response also play a role in the regulation of lipid metabolism by increasing the synthesis of certain enzymes involved in lipogenesis[16].

If adipose cells have a low lipid peroxidation level, cellular division and growth is stimulated.

During oxidative reactions, superoxide radical is generated in the body, which reacts with hydrogen peroxide and generates extremely reactive hydroxyl radicals that destroy cellular components. This situation can lead to the development of ER stress and the release of GRP78 from adipocytes[17].

The majority of recent studies maintain that chronic inflammation is a risk factor for endometrial cancer. Macrophages in the structure of adipose tissue are morphologically altered (giant cells), presenting an overactivation that is reflected by an increased secretion of cytokines (TNF-α and IL-6). These cytokines are involved in the genesis of endometrial cancer due to their effect of activating the nuclear factor kB (NF-kB), but also due to the development of ER stress with the secondary release of GRP78[18].

GRP78 is a central regulator of ER stress due to its ability to control the activation of ER transmembrane stress sensors (IRE1, Perk, and ATF6)[19,20]. Recent studies have demonstrated that GRP78 plays an important role in tumor development, progression and chemoresistance[21].

The interest in GRP78 is based on its different functions, both under normal and pathological conditions. GRP78 regulates intracellular calcium, models ER stress proteins and supports cell survival by an immediate response to insults, having anti-apoptotic properties[15]. The reduction of apoptosis allows the affected cells to survive and accumulate additional mutations.

The rapid proliferation of cancer cells requires an increase of endoplasmic reticulum activity to facilitate the folding, assembling and transmembrane transport of proteins, thus subjecting the endoplasmic reticulum to stress. The stress to which ER is subjected is the insufficient vascularization and the rapid growth of tumor cells that induce hypoxia, on one hand, and nutrient deprivation that affects protein glycosylation and ATP production, on the other hand[22,23].

Cancer cells are characterized by an altered glucose metabolism and the tumor microenvironment is marked by blood flow insufficiency and hypoxia, all these phenomena triggering endoplasmic reticulum stress. Under these conditions, tumor cells overexpress GRP78 and the pro-survival characteristics of GRP78 positively influence tumor progression and chemoresistance[24].

In our study, the plasmatic level of GRP78 had a linear positive correlation with the andoid fat and had been increased in EC. Detailed knowledge of the steps of these complex pathways, as well as cross connections between them, is necessary in order to understand the mechanisms of action of existing targeted therapy drugs and to discover new ones[25]. Determination of android fat in association with the GRP78 level could narrow down the group of patients with EC risk.

Acknowledgments: This paper was published under the frame of The European Social Fund, Human Resources Development Operational Programme 2007-2013, project no.7135/5/30.04.2014 POSDRU

References

1. Petridou E, Mantzaros C, Dessypris N,Addy C, Chransos G. Plasma Adiponectin Concentrations in Relation to Endometrial Cancer: a Case-Control study in Greece.J Clin Endocrinol Metab 2003; 3: 993-7.
2. Koong AC, Chauhan V, Romero-Ramirez L. Targeting XBP-1 as a novel anticancer strategy. Cancer Biol Ther 2006; 756-9.
3. Ciortea R, Angheluta L, Pintican D, Baltoaica R, Bucuri C, Malutan A, et al. Glucose-regulated protein 78, an important biomarker in cancer etiopathogenesis. Gineco eu 2014; 10 (3): 123-5.
4. Diehl JA, Fuchs SY, Koumenis C. The cell biology of the unfolded protein response. Gastroenterology 2011; 141(5): 38-44.
5. Matsuo K, Gray MJ, Yang DY, Srivastava SA, Tripathi PB, Sonoda LA, et al. The endoplasmic reticulum stress marker, glucose-regulated protein-78 (GRP78) in visceral adipocytes predicts endometrial cancer progression and patient survival. Gynecologic Oncology 2013; 128 (3): 552-9.
6. Bal Y, Adas M, Helvaci A. Evaluation of the relationship between insulin resistance and plasma tumor necrosis factor-alpha, interleukin-6 and C-reactive protein levels in obese women. Bratisl Lek Listy 2010; 111 (4): 200-4.
7. Allan CA, McLachlan RI. Androgens and obesity. Curr Opin Endocrinol Diabetes Obes 2010; 17 (3): 224-32.
8. Milewicz A, Jedrzejuk D, Dunajska K, Lwow F.Waist circumference and serum adiponectin levels in obese and non-obese postmenopausal women. Maturitas 2010; 65 (3): 272-5.
9. Lu JY, Huang KC, Chang LC, Huang YS, Chi YC, Su TC, et al. Adiponectin: a biomarker of obesity-induced insulin resistance in adipose tissue and beyond. J Biomed Sci 2008; 15 (5): 565-76.

10. Bray F, das Santos Silva I, Maller H, Weiderpass E. Endometrial Cancer Incidence Trends in Europe: Underlying Determinants and Prospects for Prevention. Cancer Epidemiol Biomarkers Prev 2005; 5: 1132-42.
11. Flamment M, Kammoun HL, Hainault I, Ferre P, Foufelle F. Endoplasmic reticulum stress: a new actor in the development of hepatic steatosis. Curr Opin Lipidol 2010; 2: 239-46.
12. Yang L, Xue Z, He Y, Sun S, Chen H, Qi L. A Phos-tag-based approach reveals the extent of physiological endoplasmic reticulum stress. PLoS One 2010; 5: e1162.
13. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN. et al. The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. EMBO J 2010; 29: 2082-96.
14. Langer R, Feith M, Siewert RJ, Wester HJ, Hoefler H. Expression and clinical significance of glucose regulated proteins GRP78(BiP) and GRP94 (GP96) in human adenocarcinomas of the esophagus. BMC Cancer 2008; 8: 70.
15. Pootrakul L, Datar RH, Shi SR, Cai J, Hawes D, Groshen SG, et al. Expression of stress response protein Grp78 is associated with the development of castration-resistant prostate cancer. Clin Cancer Res 2006; 12: 5987-93.
16. Daneshmand S, Quek ML, Lin E, Lee C, Cote RJ, Hawes D, et al. Glucose-regulated protein GRP78 is up-regulated in prostate cancer and correlates with recurrence and survival. Hum Pathol 2007; 38: 1547-52.
17. Lee E, Nichols P, Spicer D, Groshen S, Yu MC, Lee AS. GRP78 as a novelpredictor of responsiveness to chemotherapy in breast cancer. Cancer Res 2006; 66: 7849-53.
18. Maso LD, Augustin SA, Karalis A, Franceschi S. Circulating Adiponectin and Endometrial Cancer Risk. J Clin Endocrinol Metab 2004; 8: 1160-3.
19. Wu J, Kaufman RJ. From acute ER stress to physiological roles of the unfolded protein response. Cell Death Differ 2006; 13: 374-84.
20. Ni M, Lee AS. ER chaperones in mammalian development and human diseases. FEBS Lett 2007; 581: 3641-51.
21. Teske BF, Wek SA, Bunpo P, Cundiff JK. The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress. Mol Biol Cell 2011; 22: 4390-405.
22. Zhuang L, Scolyer RA, Lee CS. Expression of glucose-regulated stress protein GRP78 is related to progression of melanoma. Histopathology 2009; 54: 462-70.
23. Lee E, Nichols P, Groshen S. Spicer D, Lee AS. GRP78 as potential predictor for breast cancer response to adjuvant taxane therapy. Int J Cancer 2011; 128: 726-31.
24. Pfaffenbach KT, Lee AS. The critical role of GRP78 in physiologic and pathologic stress. Cell Biology 2011; 23: 150-6.
25. Markowska A, Pawałowska M, Lubin J, Markowska J.Signalling pathways in endometrial cancer. Contemp Oncol 2014; 18 (3): 143-8.