EJGO European Journal of Gynaecological Oncology

Correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma

P. Cui[1], K. Shi[2], H.X. Cui[3], L.Y. Hao[4], Y. Su[4], P.L. Li[4]

[1] Harbin Medical University, Harbin; [2] Guangzhou Women and Children's Medical Center, Guangzhou
[3] College of Environmental and Chemical Engineering, Yanshan University, Qinhuangdao (China)
[4] The 2nd Affiliated Hospital of Harbin Medical University, Harbin (China)

Summary

Aims: To investigate the correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma (EC). *Materials and Methods:* The study comprised 60 randomly selected patients diagnosed and treated at the 2nd Affiliated Hospital of Harbin Medical University from November 2011 to June 2012 for endometrial carcinoma (n = 30) and myoma of uterus (n = 30). The expression of TFAP-2β mRNA in endometrial carcinoma was analyzed by real-time reverse transcription polymerase chain reaction (RT-PCR). Body mass index (BMI), waist circumference, and venous blood samples were obtained before abdominal surgery clinically. *Results:* The expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of normal endometrium ($p < 0.05$). The expression of TFAP-2β mRNA in endometrial tissue of patients with metabolism syndrome was higher than that of lean ones ($p < 0.05$). There was no significant difference in the expression of TFAP-2β mRNA in endometrial tissue between patients with both EC and metabolism syndrome and in those with EC only. The expression levels of TFAP-2β mRNA had positive correlation with triglyceride ($r = 0.271$, $p < 0.05$) and high-density lipoprotein (HDL) ($r = 0.314$, $p < 0.05$). There was no significant correlation between the expression of TFAP-2β mRNA and CA125, fasting plasma glucose, low-density lipoprotein (LDL), waist circumference, total cholesterol, and BMI. *Conclusions:* TFAP-2β constituted promoter activity in EC and also contributed to the development of the metabolic syndrome. TFAP-2β may influence the occurrence and development of EC through regulating the expression of various adipokines and lipoprotein metabolism. Probably TFAP-2β can be a candidate tumor marker for EC.

Key words: Adipokines; Endometrial carcinoma; Metabolism syndrome; Real-time RT-PCR; TFAP-2β.

Introduction

Endometrial carcinoma (EC) is one of the three female reproductive tract malignant tumors. Its incidence has been accounted for gynecological malignant tumor first and the second most common cause of gynecologic cancer death in Europe and the United States. The incidence of EC is lower in developing countries, but the ratio of mortality to incidence is higher [1]. In recent years, economic developed rapidly of many developing countries, including China has led to the increase of its incidence. People's living habits and diet structure has been westernized, hence the incidence of obesity has also increased. Furthermore, the incidence of EC has obviously risen by informal hormone replacement therapy and other factors, including obesity, diabetes, hypertension, infertility, and menopause delay.

Obesity is associated with multiple diseases, such as breast cancer, ovarian cancer, polycystic ovary syndrome and EC [2]. Obesity plays important role in EC, but the mechanisms of action remain unclear. Adipose tissue secretes leptin and adiponectin that reportedly participates in carcinogenic processes, such as cell proliferation, angiogenesis, and insulin regulation. Metabolic syndrome, a combination of medical disorders included obesity and diabetes *et al.*, and is associated with increased endometrial cancer risk [3].

As a nuclear transcription factor, transcription factor activator protein-2 (TFAP-2) control target gene expression through closing, opening, increasing or decreasing signal transduction. It is also involved in vertebrate growth regulation, apoptosis, the occurrence and development of tumor in pathological conditions [4,5]. TFAP-2 was first purified and cloned from HeLa cells in 1987 [6]. TFAP-2 effects bidirectional regulation in tumor genesis and evolution through the regulation of tumor associated gene expression. TFAP-2 family members include TFAP-2α, TFAP-2β, TFAP-2γ, TFAP-2δ, and TFAP-2ε. TFAP2α can improve the patients with advanced bladder cancer sensitivity to cisplatin [7]. TFAP-2γ has been reported to have an effect on different target genes from Her2 breast cancer phenotype to regulate the hormone response breast cancer [8]. TFAP-2β has been associated to metabolic syndrome through playing an important role in the regulation of adipokines expression in vivo [9]. TFAP-2β is also known as a molecular marker to detect tumor and a therapeutic target for anticancer therapy [10]. The purpose of current study was to assess the association between TFAP-2β and endometrial carcinoma.

Revised manuscript accepted for publication October 8, 2014

Table 1. — *Clinical data of the patients with lean or metabolic syndrome.*

	Lean	Metabolic syndrome	*p*
Number of subjects	35	25	
Age (years)	50.49±7.78	55.84±7.14	0.042*
BMI (kg/m^2)	23.77±2.55	27.03±3.85	0.003*
Waist circumference (cm)	84.84±8.68	95.12±8.71	<0.001*
Triglycerides (mmol/L)	1.38±0.61	2.05±0.58	0.001*
Total cholesterol (mmol/L)	4.90±0.85	5.32±1.00	0.180
Fasting plasma glucose (mmol/L)	5.02±0.43	6.79±1.90	<0.001*
LDL (mmol/L)	2.89±0.62	3.09±0.79	0.399
HDL (mmol/L)	1.50±0.26	1.37±0.41	0.021*

Ages 32–82 years. Data are mean ± s.d. Metabolism syndrome was defined according to the proposal by the International Diabetes Federation. *$p < 0.05$.

Table 2. — *Clinical data of the patients with normal endometrium or endometrial carcinoma.*

	Normal endometrium	Endometrial carcinoma	*p*
Number of subjects	30	30	
Age (years)	50.83±7.71	54.60±7.39	0.151
BMI (kg/m^2)	24.87±2.41	25.38±3.95	0.807
Waist circumference (cm)	88.85±8.46	89.40±9.29	0.861
Triglycerides (mmol/L)	1.55±0.71	1.76±0.64	0.274
Total cholesterol (mmol/L)	5.11±0.96	5.05±0.93	0.850
Fasting plasma glucose (mmol/L)	5.14±0.53	6.37±1.72	0.015*
LDL (mmol/L)	2.99±0.67	2.94±0.75	0.834
HDL (mmol/L)	1.47±0.31	1.43±0.35	0.261
CA125(mmol/L)	17.39±10.32	22.54±13.30	0.003*

Ages 32–82 years. Data are mean ± s.d. Metabolism syndrome was defined according to the proposal by the International Diabetes Federation. *$p < 0.05$.

The authors hypothesized that TFAP-2β may have a high correlation with endometrial carcinoma.

Materials and Methods

Subjects

The present study including 60 patients (of the 60 patients, 30 were diagnosed and treated for EC and 30 were diagnosed and treated for myoma of uterus) were randomly selected at the 2nd Affiliated Hospital of Harbin Medical University from November 2011 to June 2012. The patients fulfilled the following inclusion criteria: (I) scheduled for surgical treatment, (II) all patients were not receiving any chemotherapy, radiotherapy, and prior targeted tumor treatment, and (III) free of severe acute illness. The endometrial tissues of patients with myoma of uterus were tested histopathologically as normal endometrium.

Body mass index (BMI) and abdominal circumference were measured in each subject. Venous blood samples were obtained before abdominal surgery. Abdominal obesity was determined by the definition criteria of the metabolic syndrome proposed by the International Diabetes Federation [11].

Endometrial tissue

Endometrial tissue biopsies were obtained during panhysterectomy, which was either laparoscopic or open-abdominal surgery. Each specimen was divided into several sections and all were immediately frozen and stored in liquid nitrogen.

Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from the frozen endometrial tissues using Trizol reagent and 1,000 ng of total RNA was reverse transcribed with a reagent kit. The primers for TFAP-2β were commercially designed and synthesized. The specific primers used of TFAP-2β were: 5'-TAAAGCGGGGAGATGGGATG-3' and 5'-GGAGAAGTGAG- GGAGGGAGAA-3'. The β-Actin mRNA was quantified for sample normalization. The thermal profile of reverse transcription consisted of 15 minutes at 37°C, five seconds at 85°C. The thermal profile of RT-PCR consisted of 30 seconds at 95°C, followed by 40 cycles of five seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C for human TFAP-2β. $2^{-\Delta\Delta CT}$ method to compare the relative expression of TFAP-2β mRNA was used. All PCRs were performed in duplicate and repeated twice.

Statistical analysis

Data were expressed as mean ± standard deviation. The Mann-Whitney Rank Sum Test was used for comparison of mean values. Pearson's correlation coefficient was used to determine the relationship between TFAP-2β gene expression and other quantitative variables. A p value of < 0.05 was considered statistically significant. Analyses were conducted using the SigmaPlot 11.0 system.

Results

Previous studies have documented that certain adipokines have been involved in tumor growth, apoptosis, and cachexia [12, 13]. In order to minimize these influences, the patients with EC who were free of acute illness or cachexia were chosen. The subject characteristics are summarized in Tables 1 and 2.

The metabolic syndrome group had a statistically higher mean age ($p = 0.042$), BMI ($p = 0.003$), waist circumference ($p < 0.001$), triglycerides ($p = 0.001$), and fasting plasma glucose ($p < 0.001$) level than the lean group. However, there were no statistically significant differences between the mean values of the lean cases and metabolism syndrome cases in terms of the total cholesterol and low-density lipoprotein (LDL).

There were no statistically significant differences between the mean values of the normal endometrium cases and EC cases in terms of the age, BMI, waist circumference, triglycerides, total cholesterol, LDL, and high-density lipoprotein (HDL). However, the EC group had a statistically higher mean of fasting plasma glucose ($p = 0.015$) and CA125 ($p = 0.003$) level than the normal endometrium group.

Expression of TFAP-2β mRNA in endometrial tissue

To date, many studies had examined the expression of AP-2 in human different tumors such as breast cancer, malignant melanoma, cervical cancer, and ovarian cancer [14,

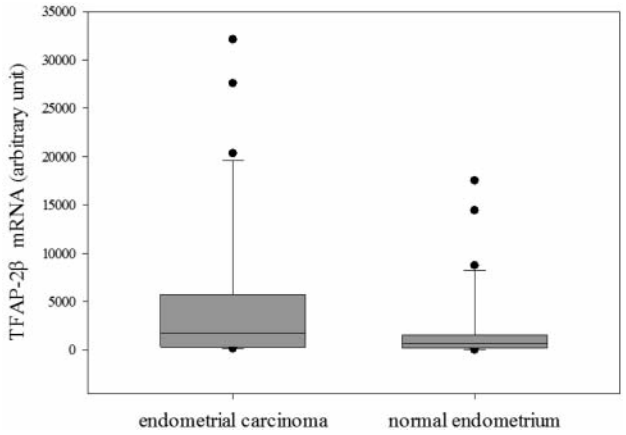

Figure 1. — Expression of TFAP-2β mRNA in endometrial tissue between EC and normal endometrium ($p < 0.05$).

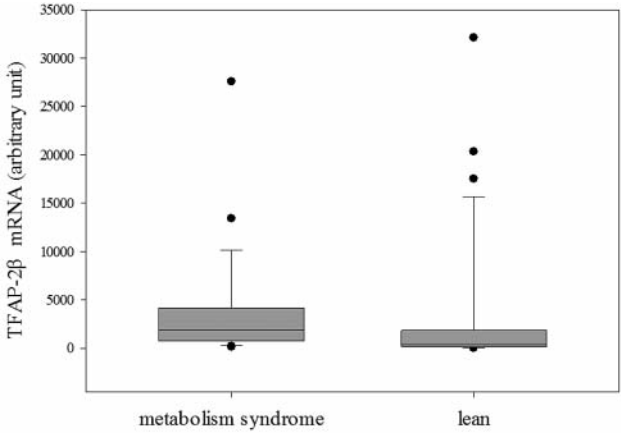

Figure 2. — Expression of TFAP-2β mRNA in endometrial tissue between metabolic syndrome and lean patients ($p < 0.05$).

15]. but their opinions are conflicting; it seems AP-2 plays a two-way regulatory role in tumorigenesis. The present study examined the role of TFAP-2β mRNA expression in EC for the first time. To test the role of TFAP-2β in EC and metabolic syndrome, the expression of TFAP-2β mRNA in endometrial tissue was examined by relative quantitative real-time PCR technology. The expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of patients with normal endometrium ($p < 0.05$, Figure 1). This result demonstrates that TFAP-2β constituted promoter activity in EC. The expression of TFAP-2β mRNA in endometrial tissue of patients with metabolic syndrome is higher than that of lean ones ($p < 0.05$, Figure 2). There was no significant difference in the expression of TFAP-2β mRNA in endometrial tissue between patients with both EC and metabolic syndrome and that with EC only (Figure 3).

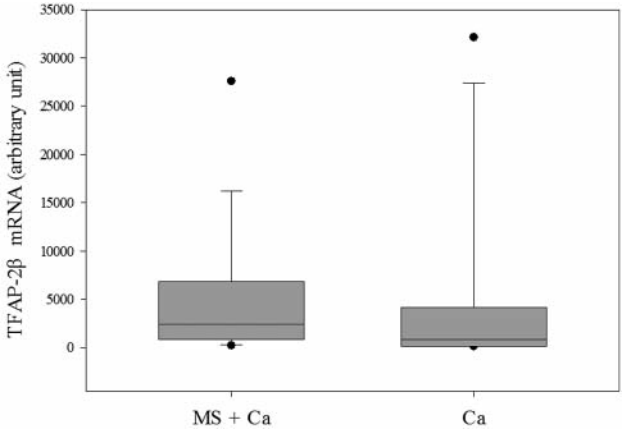

Figure 3. — Expression of TFAP-2β mRNA in endometrial tissue between the patients with both EC and metabolic syndrome and those with only EC ($p = 0.155$).

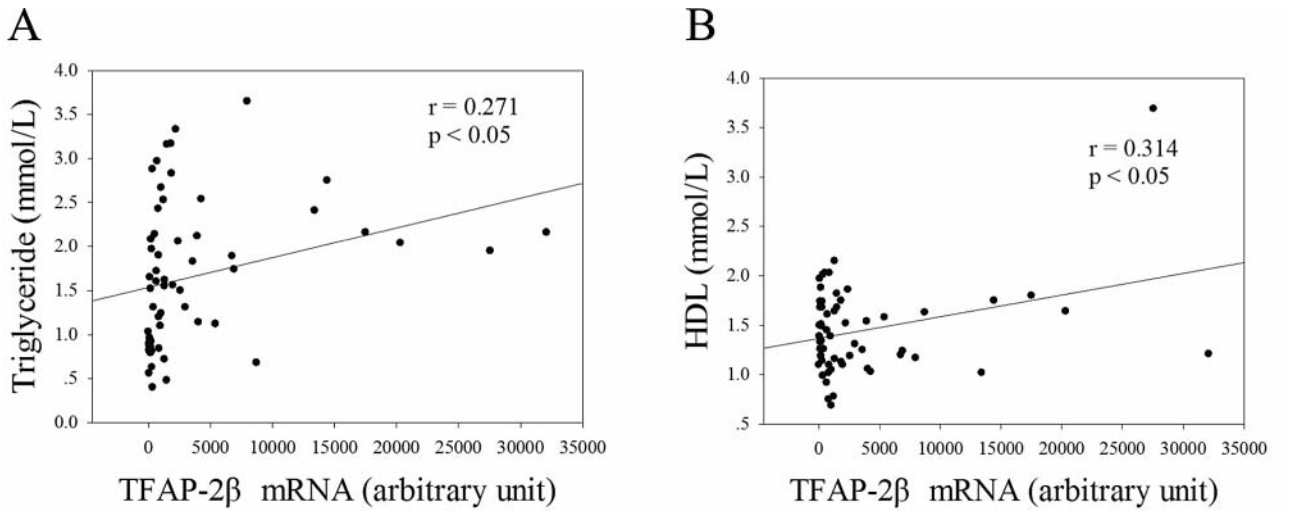

Figure 4. — Relationship between TFAP-2β mRNA expression levels in endometrial tissues and triglyceride levels and HDL levels.

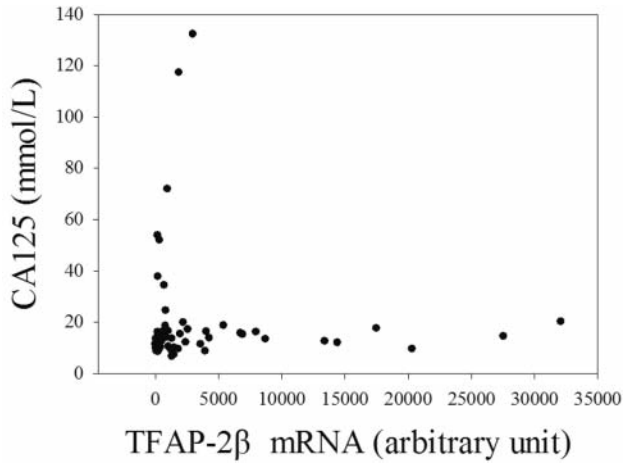

Figure 5. — Relationship between TFAP-2β mRNA expression levels in endometrial tissues and CA125 levels.

Correlation between the TFAP-2β mRNA levels and clinical data

The authors next investigated the correlation between expression of TFAP-2β mRNA in endometrial tissue and other clinical datas. TFAP-2β mRNA levels in endometrial tissue correlated positively with triglycerides ($r = 0.271$, $p < 0.05$, Figure 4a) and HDL ($r = 0.314$, $p < 0.05$, Figure 4b). CA125 had been recognized as a tumor maker and it has been associated with poor prognosis of EC [16]. It has also been verified that the EC group had a statistically higher mean CA125 ($p = 0.003$) level than the normal endometrium group in this study. Nevertheless, no significant correlation was observed between the expression of TFAP-2β mRNA and CA125 (Figure 5), fasting plasma glucose, LDL, waist circumference, total cholesterol, and BMI (Figure 6) in this experiment.

Discussion

EC is a hormone-dependent neoplasm and obesity is a well-known risk factor for it. Metabolism syndrome is used to evaluate risk factors of EC. TFAP-2β has been reported to be associated with several obesity related phenotypes [17]. The present results showed that the expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of patients with normal endometrium. It indicated that TFAP-2β might be involved in the development of EC. The expression of TFAP-2β mRNA in endometrial tissue of the patients with metabolic syndrome is higher than that of lean ones was also observed in the present experiment. TFAP-2β had a high correlation with metabolic syndrome as well. In order to eliminate the effect of metabolic syndrome that may lead to high-expression of TFAP-2β mRNA of the patients with EC, the expression of TFAP-2β mRNA in endometrial tissue was contrasted between the patients with both EC and metabolic syndrome and those with EC only. The results showed no significant difference between them. It clarified that high-expression of TFAP-2β mRNA of the patients with EC was not simply

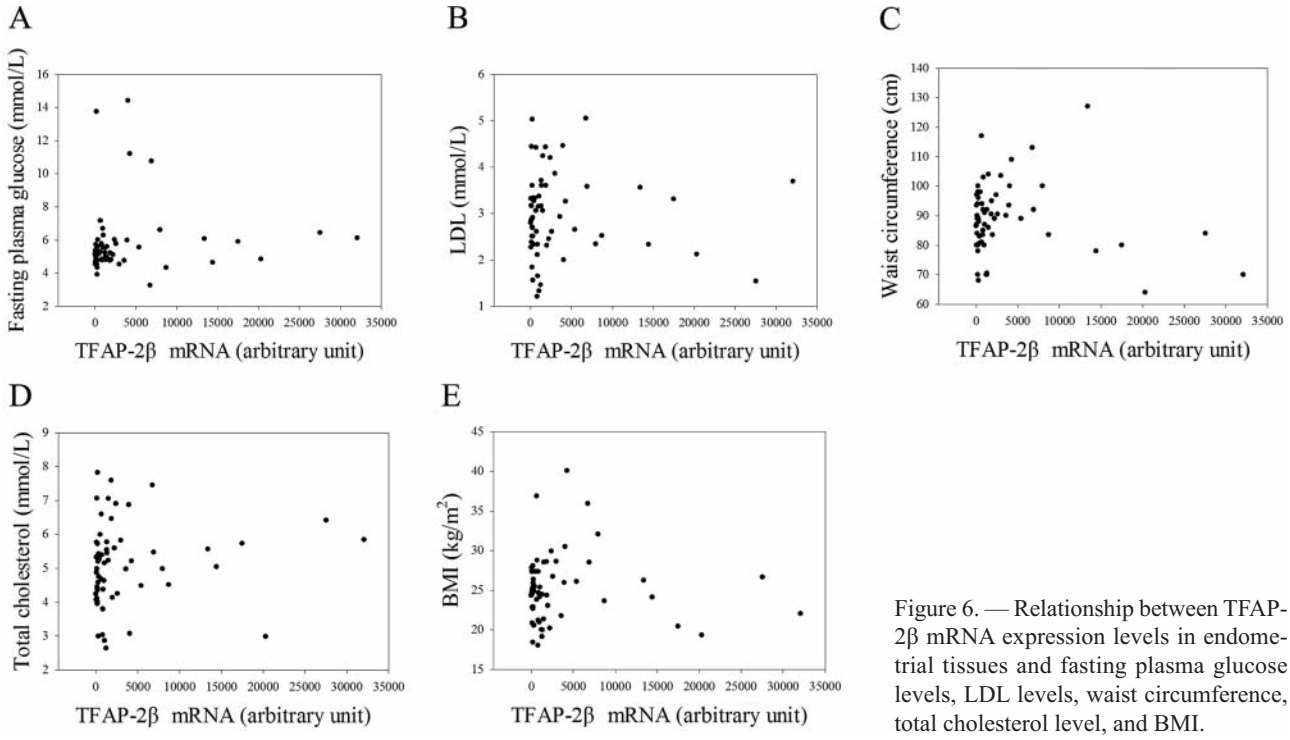

Figure 6. — Relationship between TFAP-2β mRNA expression levels in endometrial tissues and fasting plasma glucose levels, LDL levels, waist circumference, total cholesterol level, and BMI.

due to a metabolic syndrome. It demonstrated that TFAP-2β may not only play an advanced role in EC but may also contribute to the development of the metabolic syndrome.

Systemic metabolic derangement is closely related to fat distribution [17]. Numerous studies have shown the adipokines level in endometrial carcinoma, especially leptin and adiponectin. Leptin was involved in proliferative processes of the endometrium and positively associated with EC [18, 19]. Low serum levels of adiponectin were independently associated with EC [20, 21]. Ugi *et al.* comprised 81 individuals and then suggested that TFAP-2β played a major role in the regulation of various adipokines and TFAP-2β correlated negatively with adiponectin and leptin [9]. Therefore, low serum levels of adiponectin should accompany high-expression of TFAP-2β. It corresponded to the present results that high-expression of TFAP-2β promoted tumorigenesis of EC. It can be reasonably inferred that TFAP-2β may effect the development of EC through regulating the expression of various adipokines such as leptin and adiponectin.

Detailed analysis of lipid components showed that there is a consistent relation between TG levels and EC risk [22]. Meanwhile, the metabolic pathway and its relation to EC risk are still unclear. The present study showed that the TFAP-2β mRNA levels in endometrial tissue correlated positively with serum triglycerides. This result indicated that TFAP-2β might lead to elevated triglycerides. The present results also showed that TFAP-2β mRNA levels in endometrial tissue correlated positively with HDL, which no study has yet reported. It indicates that TFAP-2β may regulate the lipoprotein metabolism, such as triglycerides and HDL. Therefore, TFAP-2β may influence the tumorigenesis of EC through regulating the expression of triglycerides and HDL.

No reliable tumor marker has been approved for EC. Serum CA125 was introduced as a circulating antigen in epithelial ovarian cancer first and it has been clinically widely used in EC [23]. The key tumor markers of EC are remaining debatable. The value of CA125 in EC has been investigated in many studies; however, results have been conflicting. Sood *et al.* [16] have shown that preoperatively elevated CA125 would contribute to poor survival and several authors have also found that higher serum CA-125 levels correlated with stage, histopathology, and LN metastasis factors [24, 25]. Other reports have shown that CA125 levels and development of disease are not correlated [26]. Therefore, CA125 has been considered as a predictor of worse prognosis but not a reliable tumor marker for endometrial carcinoma. Our study has found that the endometrial carcinoma group had a statistically higher mean CA125 ($p=0.003$) level than the normal endometrium group as Sood et al showed. Meanwhile, our result also showed that CA125 was not related to TFAP-2β mRNA expression in EC. TFAP-2β may predict the occurrence of endometrial carcinoma.

In conclusion, this study demonstrated that TFAP-2β constituted promoter activity in EC and also contributed to the development of the metabolic syndrome. TFAP-2β may influence the occurrence and development of EC through regulating the expression of various adipokines and lipoprotein metabolism. Most likely TFAP-2β can be a candidate tumor marker for EC. Future research work will focus on upstream regulation gene of induced HDL and triglycerides to study biologic effect of TFAP-2β on endometrial carcinogenesis.

Acknowledgement

The authors thank Professor Hong Ling from Microbiology Laboratory of Harbin Medical University for all her kindness and technical help.

References

[1] Parkin D.M., Bray F., Ferlay J., Pisani P.: "Global cancer statistics, 2002". *CA Cancer J. Clin*., 2005, *55*, 74.

[2] Huff J.: "IARC monographs, industry influence, and upgrading, downgrading, and under-grading chemicals: a personal point of view. International Agency for Research on Cancer". *Int. J. Occup. Environ. Health*, 2002, *8*, 249.

[3] Friedenreich C.M., Biel R.K., Lau D.C., Csizmadi I., Courneya K.S., Magliocco A.M., *et al.:* "Case-control study of the metabolic syndrome and metabolic risk factors for endometrial cancer". *Cancer Epidemiol. Biomarkers Prev*., 2011, *20*, 2384.

[4] Eckert D., Buhl S., Weber S., Jager R., Schorle H.: "The AP-2 family of transcription factors". *Genome Biol*., 2005, *6*, 246.

[5] Mitchell P.J., Timmons P.M., Hebert J.M., Rigby P.W., Tjian R.: "Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis". *Genes Dev*., 1991, *5*, 105.

[6] Mitchell P.J., Wang C., Tjian R.: "Positive and negative regulation of transcription in vitro: enhancer-binding protein AP-2 is inhibited by SV40 T antigen". *Cell*, 1987, *50*, 847.

[7] Nordentoft I., Dyrskjøt L., Bødker J.S., Wild P.J., Hartmann A., Bertz S., *et al.:* "Increased expression of trans criptionfactor TFAP2alpha correlates with chemosensitivity in advanced bladder cancer". *BMC Cancer*, 2011, *11*, 135.

[8] Woodfield G.W., Chen Y., Bair T.B., Domann F.E., Weigel R.J.: "Identification of primary gene targets of TFAP2C in hormone responsive breast carcinoma cells". *Genes Chromosomes Cancer*, 2010, *49*, 948.

[9] Ugi S, Nishio Y, Yamamoto H., Ikeda K., Kobayashi M., Tsukada S., *et al.:* "Relation of the expression of transcriptional factor TFAP2B to that of adipokines in subcutaneous and omental adipose tissues". *Obesity (Silver Spring)*, 2010, 18, 1277.

[10] Deng W.G., Jayachandran G., Wu G., Xu K., Roth J.A., Ji L.: "Tumor-specific activation of human telomerase reverses transcriptase promoter activity by activating enhancer-binding protein-2beta in human lung cancer cells". *J. Biol. Chem*., 2007, *282*, 26460.

[11] Alberti K.G., Zimmet P., Shaw J.: "The metabolic syndrome—a new worldwide definition". *Lancet*, 2005, *366*, 1059.

[12] Cowey S., Hardy R.W.: "The metabolic syndrome: A high-risk state for cancer?" *Am. J. Pathol.*, 2006, *169*, 1505.

[13] Kerem M., Ferahkose Z., Yilmaz U.T., Pasaoglu H., Ofluoglu E., Bedirli A., *et al.:* "Adipokines and ghrelin in gastric cancer cachexia". *World J. Gastroenterol*., 2008, *14*, 3633.

[14] Hietala K.A., Kosma V.M., Syrjanen K.J., Syrjanen S.M., Kellokoski J.K.: "Correlation of MIB-1 antigen expression with transcription factors Skn-1, Oct-1, AP-2, and HPV type in cervical intraepithelial neoplasia". *J. Pathol*., 1997, *183*, 305.

[15] Anttila M.A., Kellokoski J.K., Moisio K.I., Mitchell P.J., Saarikoski S., Syrjänen K., Kosma V.M.: "Expression of transcription factor AP-2alpha predicts survival in epithelial ovarian cancer". *Br. J. Cancer*, 2000, *82*, 1974.

[16] Sood A.K., Buller R.E., Burger R.A., Dawson J.D., Sorosky J.I., Berman M.: "Value of preoperative CA 125 level in the management of uterine cancer and prediction of clinical outcome". *Obstet. Gynecol*., 1997, *90*, 441.

[17] Wajchenberg B.L.: "Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome". *Endocr. Rev*., 2000, *21*, 697.

[18] Petridou E., Belechri M., Dessypris N., Koukoulomatis P., Diakomanolis E., Spanos E., Trichopoulos D.: "Leptin and body mass index in relation to endometrial cancer risk". *Ann. Nutr. Metab*., 2002, *46*, 147.

[19] Cymbaluk A., Chudecka-Glaz A., Rzepka-Gorska I.: "Leptin levels in serum depending on Body Mass Index in patients with endometrial hyperplasia and cancer". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2008, *136*, 74.

[20] Dal Maso L., Augustin L.S., Karalis A., Talamini R., Franceschi S., Trichopoulos D., *et al.*: "Circulating adiponectin and endometrial cancer risk". *J. Clin. Endocrinol. Metab*., 2004, *89*, 1160.

[21] Soliman P.T., Wu D., Tortolero-Luna G., Schmeler K.M., Slomovitz B.M., Bray M.S., *et al.:* "Association between adiponectin, insulin resistance, and endometrial cancer". *Cancer*, 2006, *106*, 2376.

[22] Seth D., Garmo H., Wigertz A., Holmberg L., Hammar N., Jungner I., *et al.:* "Lipid profiles and the risk of endometrial cancer in the Swedish AMORIS study". *Int. J. Mol. Epidemiol. Genet*., 2012, *3*, 122.

[23] Niloff J.M., Klug T.L., Schaetzl E., Zurawski V.R. Jr., Knapp R.C, Bast R.C. Jr.: "Elevation of serum CA125 in carcinomas of the fallopian tube, endometrium, and endocervix". *Am. J. Obstet. Gynecol*., 1984, *148*, 1057.

[24] Dotters D.J.: "Preoperative CA 125 in endometrial cancer: is it useful?" *Am. J. Obstet. Gynecol*., 2000, *182*, 1328.

[25] Yildiz A., Yetimalar H., Kasap B., Aydin C., Tatar S., Soylu F., Yildiz F.S.: "Preoperative serum CA 125 level in the prediction of the stage of disease in endometrial carcinoma". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2012, *164*, 191.

[26] Lo S.S., Cheng D.K., Ng T.Y., Wong L.C., Ngan H.Y.: "Prognostic significance of tumour markers in endometrial cancer". *Tumour Biol*., 1997, *18*, 241.

Address reprint requests to:
K. SHI, M.D.
Department of Obstetrics and Gynaecology
Guangzhou Women and Children's Medical Center
9 Jinsui Road, Guangzhou 510623 (China)
e-mail: shikun28@hotmail.com