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EPIGENETIC MECHANISMS OF TNFα ACTIVATION IN PATIENTS WITH ENDOMETRIAL CANCER

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Abstract

Timely identification of risk groups, early signs of the disease onset and recurrence is an important aspect in management of endometrial cancer (EC) patients. There is an active search for early diagnosis new markers, detection of relapses and EC postoperative monitoring. *Tumor necrosis factor alpha (TNFα)* is a cytokine involved in the pathogenesis of various forms of cancer, as well as associated with chronic inflammation, obesity. **The objective** is to study TNFa gene methylation in EC and the possibility of its use as a marker for forecasting, monitoring, and EC risk development. **Materials and methods**. DNA methylation was determined by pyrosequencing in endometrial specimens taken from 10 patients with verified endometrial hyperplasia and 13 patients with EC when performing hysteroscopy with endometrial biopsy or endometrial curettage. **Results and discussion.** The total degree of methylation of the TNFα gene DNA promoter in the samples under study in the patients with simple and / or complex nonatypical endometrial hyperplasia was 62.6 ± 12.8%, which is higher than in EC patients (34.7 ± 8.8%). **Conclusions.** The results obtained showed the involvement of epigenetic mechanism associated with hypomethylation of the TNFα gene promoter, which can lead to the activation of the TNFα gene in EC. Determination of methylation DNA promoter of the TNFα gene and its amount can be used as a potential prognostic and diagnostic marker of EC.

Key words: methylation, TNFα, endometrial cancer, diagnosis.

Introduction. Endometrial cancer (EC) has top billing in cancer diseases of the female genital. Its treatment often includes surgical, radiotherapeutical and chemotherapeutic methods, which require constant monitoring of effectiveness. An important aspect of EC patients management is timely identification of risk groups, early signs of the onset and recurrence of the disease. Currently proteomic tumor-associated markers detected by enzyme immunoassay method - cancer antigen 125 (CA125) and human epididymis protein 4 (HE4)- is used. With a high level of sensitivity, CA125 has a low level of specificity regarding tumor localization. HE4 is a tumor marker with higher sensitivity and specificity for the diagnosis of tumors of the pelvic organs compared to CA125. It should be noted that the diagnostic capabilities of both markers, HE4 and CA125, are limited [1]. Therefore, an active search for new markers for early diagnosis, detection of relapses and postoperative monitoring of EC is currently underway. It has been proven that many forms of tumor transformation are associated with chronic inflammation, obesity, and diabetes. Cytokines have attracted keen attention of researchers, which is associated with their participation in the pathogenesis of various forms of cancer [2]. One such cytokine is tumor necrosis factor alpha (TNFα), associated with chronic inflammation, obesity and cancer.

TNF can play a dual role in tumor biology. It is a cytokine with well-known anti-cancer properties, which can also contribute to the development and progression of cancer [3]. The literature describes that in patients with a diagnosis of lung cancer in the TNF network hypomethylated genes were cytokines CCL3, CCL4, CCL7, CCL8, CCL22, IL21, IL17A, EBI3, which can either stimulate or inhibit the growth and progression of the tumor [4]. Preservation of high expression of TNFα and proinflammatory cytokines leads to tissue damage in the inflammatory focus, including stimulation of metalloproteinases. production In addition, TNFα is involved in the regulation of the proapoptotic Bcl2 gene. The involvement of TNFα in the regulation of various cellular pathways makes it a significant target for studying it as a marker for diagnosis, prognosis and a possible target for the therapy of EC.

Objective: to study the methylation of the TNFa gene in EC and the possibility of its use as a marker for forecasting, monitoring, of EC's risk development.

Materials and methods.

Endometrial samples were taken from the surgical material of 13 patients (42–79 years old) with a morphologically verified EC, as well as uterine mucosa with simple and / or complex nonatypical endometrial hyperplasia of 10 women (aged 27-43 years old).

The women under study were admitted in a planned or urgent manner to the gynecological department of the Multidisciplinary Medical Center (University Clinic No 1) of the Odessa National Medical University for performing hysteroscopy with targeted biopsy, resection of the endometrium (if medically indicated) or fractional treatment-diagnostic curettage of the uterus walls. Indications for treatment and diagnostic operations in patients with simple and / or complex endometrial hyperplasia, EC were as following: abnormal uterine bleeding, endometrial hyperproliferative process according to the results of ultrasound examination of the pelvic organs (in order to clarify the pathological diagnosis).

To study the methylation of the TNFα gene in EC patients and patients with simple and / or complex non-typical hyperplasia of the endometrium, DNA from tissue samples was isolated using the QIAamp DNA Mini Kit (Qiagen). The DNA concentration was determined spectrophotometrically on NanoDrop spectrophotometer and adjusted to concentration of 1 μg / ml in all samples. Bisulfite treatment of DNA isolated was performed with EpiTect Plus Bisulfite Kits kit (Qiagen). DNA amplification was performed with HotStarTaq DNA Polymerase kit (Qiagen) according to the program (Table 1): 95ºC - 15 min; 95ºC - 30 sec, annealing of primers 52º C - 30 sec, elongation 72ºC - 10 min. Pyrosequencing was performed with PyroMark Gold Q24 reagent kits (Qiagen) on a PyroMark Q24 instrument in the laboratory of molecular pathology of the Institute of Pathology (Erlangen, Germany). The content of methylated DNA in the sample was evaluated with PyroMark CpG software 2.01 program. Statistical data processing was performed using the program Statistica, Versia 10.

Table 1. Primers for DNA methylation analysis of the TNFα gene

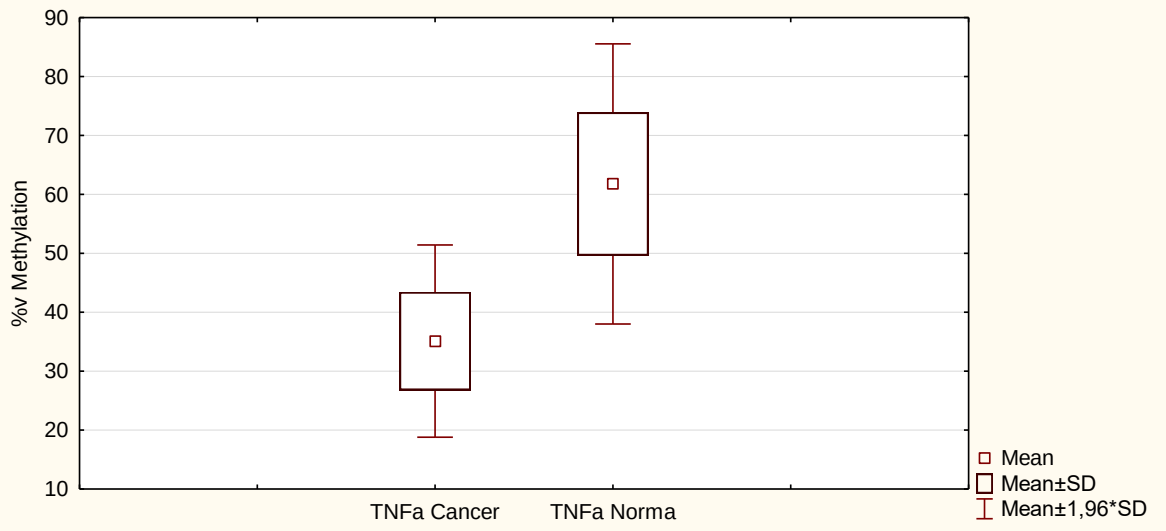
Gene name	Primer	T, ^{0}C
TNFα	F-[Biotin]GAGTGTGAGGGGTATTTTTGATG	52
TNFα	R-GCAACCATAATAAACCCTACACCTTC	
TNFα	Seq-AAACCCTACACCTTCTATCT	*LINE1*

Results and discussion.

DNA methylation of the TNFα gene promoter was studied in 13 patients with EC and 10 women with simple and / or complex nonatypical endometrial hyperplasia. Four methylation sites in the proximal promoter of the TNFα gene were analyzed.

The results of the study showed that the total degree of TNFα gene promoter methylation in the samples under study in patients with simple and / or complex nonatypical endometrial hyperplasia was 62.6 ± 12.8% which was higher than in patients with EC, where this indicator was 34.7 ± 8.8% (Fig. 1). That is, in the endometrial tissue with simple and / or complex non-

atypical hyperplasia, the content of methylated DNA of the TNFα gene is 62.6%. In EC patients it was equal to 34.7%. The decrease in the content of methylated DNA of TNFα gene promoter in EC samples compared with samples of simple and complex nonatypical endometrial hyperplasia was statistically significant, $p \leq 0.01$.



* TNFα Cancer - RE patients

* TNFα Norma - patients with simple and / or complex nonatypical endometrial hyperplasia

Fig. 1. DNA methylation of the TNFα gene promoter in re-tissue samples and simple and / or complex nonatypical endometrial hyperplasia.

Reducing DNA methylation of TNFα gene promoter leads to an increase in TNFα expression in endometrial tissue and may contribute to the manifestation of various direct and mediated cellular effects associated with the participation of TNFα in the regulatory pathways responsible for proliferation, apoptosis, proinflammatory and cytotoxic effects.

TNFα isimmune system key cytokine, which initiates and stimulates inflammation, and the latter in certain conditions can lead to the development of chronic inflammatory diseases and uncontrolled cell growth. Activation of the NFκB pathway in epithelial cells, macrophages, neutrophils is a key effect of TNF, leading, on the one hand, to increased production of pro-inflammatory cytokines, and, on the other hand, to the production of iNOS, COX-2 and NOX subunits, thereby activating NADPH oxidase, which leads to the production of reactive oxygen species (ROS). As already known, free hydroxyl superoxide radicals can contribute to DNA and RNA damage, oxidize enzymes, proteins, cell membrane lipids, increase cell proliferation, thereby contributing to the growth and progression of the tumor.

Conclusions.

The results obtained showed epigenetic mechanism involvement, which is associated with hypomethylation of TNFα gene promoter, which can lead to the activation of TNFα gene in EC. Estimation of the amount of methylation DNA promoter of the TNFα gene can be used as a EC's potential prognostic and diagnostic marker.

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