The Expression Analysis of Specific Genes in Ovarian Cancer

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Araştırma Makalesi/Research Article
DOI: *10.59312/ebshealth.1366847*
Geliş Tarihi / Received: *26.09.2023*
Kabul Tarihi / Accepted: *29.10.2023*

ABSTRACT

Aim: Ovarian cancer (OC) is the most lethal gynecologic malignancy and frequently diagnosed at an advanced stage because of the inadequate number of biomarkers. Therefore, identification of OC specific biological markers is a vital step for diagnosis and treatment response. Our goal is to examine functional gene sets which are possibly markers for ovarian cancer and their expression profiles in OC patients. We also aim to determine the potential genes for therapeutic targets for OC patients.

Method: The expression levels of seven genes (FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, and VEGFA) were identified by qRT-PCR. The tumor-free control group consisted of total abdominal hysterectomy (n=1) and bilateral salpingo-oophorectomy (n=9) patients who underwent gynecologic procedures. High-grade serous OC epithelial samples (n=10) were used for the experiment group.

Results and Conclusions: According to the qRT-PCR data, there is an increased expression of FOS (p=0.0089), MMP-9 (p=0.0029), VEGFA (p=0.0434) and decreased expression of FOSL2 (p=0.0271), JUN (p=0.0041), TIMP-2 (p=0.0062). In conclusion, the results can indicate the new perspective for OC pathogenesis and treatment. For future studies, these genes can be used in personalized diagnosis and therapy of OC.

Keywords: Ovarian cancer, gene expression, biomarker

Over Kanserinde Belirli Genlerin Anlatım Analizi

ÖZ

Amaç: En ölümcül jinekolojik malignite olan over kanseri (OK), tanısal ve prognostik biyobelirteçlerin eksikliği nedeniyle genellikle ileri evrede teşhis edilir. Bu nedenle, OK'ye özgü biyolojik belirteçlerin tanımlanması, teşhis ve tedavi yanıtı için çok önemli bir adımdır. Amacımız, OK hastalarında over kanseri için potansiyel belirteç olan fonksiyonel gen setlerini ve ekspresyon profillerini incelemektir. Ayrıca OK hastaları için olası terapötik hedefler olabilecek genlerin potansiyelini belirlemeyi de amaçlarımızdandır.

Yöntem: qRT-PCR kullanılarak yedi genin (FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2 ve VEGFA) ekspresyon profilleri belirlenmiştir. Kontrol grubu, tümör oluşumu gözlenmeyen, jinekolojik prosedür uygulanan total abdominal histerektomi (n=1) ve bilateral salpingo-ooferektomi (n=9) hastalarından oluşturulmuştur. Deney grubu için yüksek dereceli seröz OC epitel örnekleri (n=10) kullanılmıştır.

Bulgular ve Sonuçlar: qRT-PCR verilerine göre FOS (p=0,0089), MMP-9 (p=0,0029), VEGFA (p=0,0434) ekspresyonunda artış ve FOSL2 (p=0.0271), JUN (p=0.0041) ve TIMP-2 (p=0.0062) ekspresyonunda azalma tespit edilmiştir. Sonuç olarak, veriler OK patogenezi ve tedavisi ile ilgili yeni yaklaşımlar geliştirilmesini sağlayacaktır. Aday genler, gelecekte OK için kişiselleştirilmiş tanı ve tedaviyi geliştirebilecektir.

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This study was presented as an oral presentation at the 3rd International Multidisciplinary Cancer Research Congress (7-10 September 2023

INTRODUCTION

Ovarian cancer (OC) is one of the most frequently diagnosed malignant carcinomas and is the leading cause of gynecological cancer-related death (Singh and Som, 2021; Zheng et al., 2019; Torre et al., 2018). Because of asymptomatic characteristics, more than 70% of OC cases are detected at the advanced stages (Yang et al., 2020; Torre et al., 2018). Low treatment efficiency is caused by several factors, including diagnosis at advance stages, inadequate number of biomarkers, the development of drug resistance, and phenotype heterogeneity (Alshamrani AA., 2020). Identification of prognostic biomarkers and the development of personalized treatment for OC patients are significant due to the difficulties in early and effective diagnosis and the heterogeneous prognosis of OC (Liu et al., 2022; Yang et al., 2020). The gene expression profiles of tissue or liquid biopsy samples are widely used for the classification, diagnosis, or prognosis of different diseases including cancer (Gumusoglu-Acar et al., 2023; Apostolou et al., 2019; Gunel et al., 2019). Currently, studies have focused on the risk factors underlying the etiology of OC by gene expression analysis. The dysregulation of the several genes is shown to be associated with OC (Ono et al., 2000). There has been an increasing interest in screening differentially expressed genes using various expression-based technologies (Olbromski et al., 2022; Rutter et al., 2019; Gunel et al., 2019; Narrandes and Xu, 2018). In this study, seven genes (*FOS* (Olbromski et al., 2022), *FOSL2* (Li et al, 2022), *JUN* (Olbromski et al., 2022), *MMP-2* (Kicman et al., 2022; Zeng et al. 2020), *MMP-9* (Kicman et al., 2022; Zeng et al. 2020), *TIMP-2* (Escalona et al., 2020), *VEGFA* (Li et al, 2020)) that are effective in the pathogenesis of OC were selected based on the literature. Therefore, this study aimed to screen gene expressions by qRT-PCR validation to generate novel knowledge related to OC.

METHODS

Sample Collection

Tissue samples of high-grade serous ovarian cancer (HGSOC) patients (n=10) and individuals in the control group (n=10) were recruited from surgical specimens at the Department of Obstetrics and Gynecology, Istanbul Medical Faculty at Istanbul University. Normal ovary tissue samples were recruited from non-tumour-related oophorectomies performed in non-cancerous patients. OC tissues were obtained from patients diagnosed with primary HGSOC, and not previously undergone chemotherapy treatment or surgery. The methodology and design of study, and the sample collection steps are ethically approved by the Istanbul University Faculty of Medicine Clinical Researches Ethics Committee (Permission No: 2014/1175) on 08.08.2014. Each tissue sample was preserved in RNA Later Solution and stored at − 80 °C.

Total RNA Isolation

Total RNA was isolated from the tissue using the RNeasy® Plus Mini Kit (QIAGEN, GmbH) by following the manufacturer's instructions. Total RNA concentrations were measured by NanoDrop IMPLEN P-Class (Thermo Fisher Scientific, Inc.). Total RNA was diluted to a concentration of 100 ng/µl for reverse transcription. The isolated total RNA was stored at −80°C.

Reverse Transcription and qRT-PCR

Complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Inc.) by following the manufacturer's instructions. The Agilent SureCycler 8800 Thermal Cycler (Agilent, Santa Clara, Inc.) performed all the reverse transcription reactions. The qRT-PCR was performed with CFX96 C1000 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) using SsoAdvanced Universal SYBR Green PCR Master Mix (Bio-Rad Laboratories, Inc.) following the manufacturer's guidelines. GAPDH gene was used as an internal control for gene expression normalization for qRT-PCR. qRT-PCR results were analyzed by comparative threshold cycle (Ct) method 2^(−ΔΔCt), and were expressed as the relative quantification (RQ) values.

Statistical Analysis of qRT-PCR

Statistical analysis was performed using the GraphPad Prism (v.9). P-values less than <0.05 were considered statistically significant for all tests. The gene expression difference between OC patients and controls is analyzed by Mann–Whitney U test using the 2^(−ΔΔCt) values of each group. P-value, CI, and standard deviations (SD) were calculated using Ct values obtained from qRT-PCR results. For sensitivity and specificity tests, receiver operating characteristic (ROC) curves and areas under the ROC curves (AUC) were performed. The cut-off for assuming the diagnostic value for differentiating between control and patient outcomes was established as the AUC value 0.5.

FINDING AND DISCUSSION

Clinical characteristics

The demographic and clinical characteristics of patients are shown in **Table 1**. A total of 10 subjects (median age, 50) with HGSOC patients and 10 with control (median age, 56) were enrolled in the current study. In our study, all patients had undergone primary cytoreductive surgery, and none of the patients had received neoadjuvant chemotherapy or had undergone assisted reproduction.

Table 1. Clinical characteristics of the study sample.

	HGSOC (*n*=10)	**Control (*n*=10)**
Mean Age	50	56
Mean of CA-125 (IU/mL)	1697,67	N/A
Alcohol, n(%)	0(%0)	N/A
Smoking, n(%)	1 (%10)	N/A
Histological Types and Stages	5 cases are stage 3C grade 3 serous cancer, 2 cases are stage 3B grade 3 serous cancer, 1 case is 2A endometrioid, 1 case is 2B grade 1 serous cancer, 1 case is serous cancer with unknown grade	5 cases are leiomyomas, 3 cases are uterine polyps, 1 case is cystocele, 1 case is adnexal mass
The Number of Patients with Metastasis or Other diseases Occurred After Sample Collection	9 patients developed metastasis	9 cases are total abdominal hysterectomy and bilateral salpingo-oophorectomy, 1 case is total abdominal hysterectomy
Survival	6 patients died	N/A

1. *N/A: Not available*

The expression analysis of specific gene set

The expression analysis of mRNA was performed on all ovary tissue samples. *FOS*, *MMP-9* and *VEGFA* showed an increased expression and *FOSL2*, *JUN* and *TIMP-2* showed a decreased expression, **Fig. 1**. Except *MMP-2*, all of the genes were significant **(Table 2)**. The highest expressed gene was *MMP-9* (*P*=0.0029).

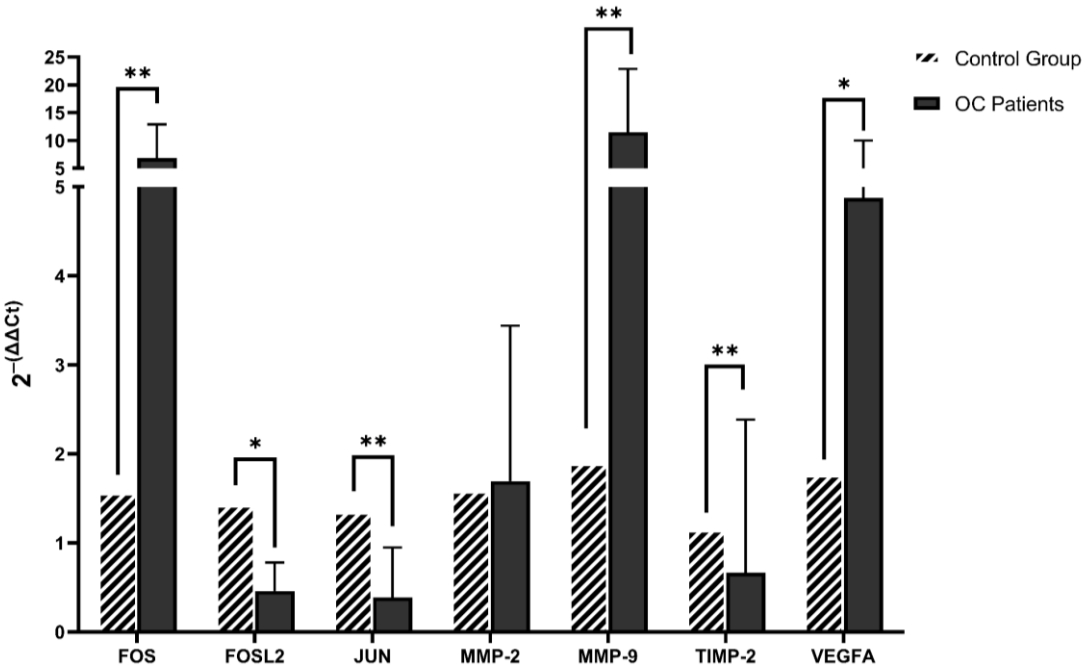

Fig 1. Relative expression analysis of tissue mRNAs. Significant (*P*<0.05) genes of interest. *P*-value ranges: 0.001–0.01 (very significant)**; 0.01–0.05 (significant)*; ≥ 0.05 (not significant).

Table 2. Average of patients and controls FC value.

GENE	PATIENT	CONTROL	P-VALUES
FOS	6.8	1.5	0.0089
FOSL2	0.4	1.4	0.0271
JUN	0.3	1.3	0.0041
MMP-2	1.7	1.5	0.1712*
MMP-9	11.5	1.9	0.0029
TIMP-2	0.6	1.1	0.0062
VEGFA	4.8	1.7	0.0434

**: Not significant*

According to ROC curve analysis, the discriminability of the genes was determined. Highest AUC was 0.88 for *MMP-9*, and lowest AUC was 0.78 for *VEGFA*. ROC Curves can be seen in **Fig 2**.

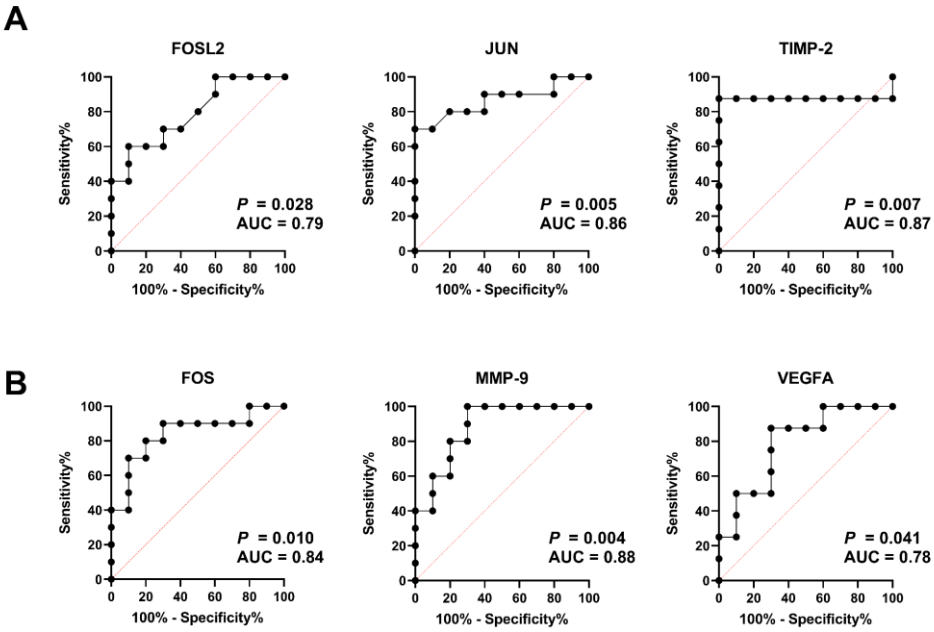

Fig 2. Results of receiver operating characteristic (ROC) parameters for increased and decreased putative genes OC patients were shown as A (decreased) and B (increased).

DISCUSSION

The prognosis prediction and the designing therapeutic approaches for ovarian cancer are still difficult because of the complex pathophysiology of OC. Therefore, it is essential and urgent to investigate validated biomarkers and certain molecular pathways for the early diagnosis, treatment, and prognosis of OC. Thus, in the present study, seven genes (*FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, VEGFA*) expressions were determined with qRT-PCR to investigate whether common pathways influence OC pathogenesis.

c-FOS is a member of the FOS protein family (c-Fos, FosB, Fra1, and Fra2) that forms the AP-1 transcription factor and its overexpression is positively associated with growth in many tumors, including ovarian cancer (Bejjani et al., 2019). Our results regarding the significantly increased expression of *c-FOS* (*P*= 0.0089) is consistent with the current literature (Olbromski et al., 2022). **c-JUN** is a member of the JUN multigene protein family (c-Jun, JunB, JunD) (Bejjani et al., 2019) and an important member of AP1 transcription factor, which has roles in many molecular processes like cell survival proliferation and differentiation. In our study, gene expression analysis showed a statistically significant decrease in *c-JUN* (*P*=0.0041). Olbromski et al. (2022) supported our results in which a significant decrease in mRNA expression level of *c-JUN*.

MMP-2 and ***MMP-9*** are genes of the gelatinase family generally taking role on denaturing and cleaving type IV collagen and gelatine. Their expressions are significantly upregulated in cancer cell migration or invasion processes (Kicman et al., 2022; Zeng et al. 2020). It is indicated that *MMP-9* is a potential diagnostic serum marker for ovarian cancer and a potential therapeutic target for ovarian cancer therapy (Zhang and Chen, 2017). In our results, the level of *MMP-9* was dramatically increased (*P*=0.0029). Although it is not statistically significant, we found that *MMP-2* is upregulated in OC patients, which is consistant with Poon et al.'s study that found significantly increased *MMP2* level in OC (Poon et al., 2011).

The ***TIMP-2*** (Tissue Inhibitor of Metalloproteinases-2) gene has been implicated in the progression and metastasis of ovarian cancer. Decreased expression of *TIMP-2* in ovarian cancer cells is associated with increased tumor growth, invasion, and metastasis, as well as poor patient prognosis and survival rates. *TIMP-2* is also found as differentially expressed (*P*=0.0062) in our results. Moreover, it is suggested that *TIMP-2* expression may serve as a robust biomarker for prognosis in ovarian cancer (Escalona et al., 2020).

VEGF signaling pathways have a key function in tumor angiogenesis and lymphangiogenesis (Saharinen et al., 2011). VEGF-A is one form of the VEGF family and has an important role in vasculogenesis and angiogenesis by regulating the activity of endothelial cells (Jang et al, 2017; Li et al, 2020). *VEGFA* can be one of the most promising angiogenic factors for clinical use as a prognostic marker of ovarian cancer (Sopo et al, 2019). Significant upregulation of *VEGFA* expression shown in our results (*P*= 0.04) supports all these findings and is associated with HGSOC.

CONCLUSION

In conclusion, our research identifies important candidate genes in pathways that are dysregulated and linked to OC. The findings of this study can improve our understanding of the molecular etiology of OC and can be applied to further studies on OC-related biomarkers and drug development.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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