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ORIGINAL ARTICLE

# Molecular markers associated with nonepithelial ovarian cancer in formalin-fixed, paraffin-embedded specimens by genome wide expression profiling

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**Abstract** Nonepithelial ovarian cancer (NEOC) is a rare cancer that is often misdiagnosed as other malignant tumors. Research on this cancer using fresh tissues is nearly impossible because of its limited number of samples within a limited time provided. The study is to identify potential genes and their molecular pathways related to NEOC using formalin-fixed paraffin embedded samples. Total RNA was extracted from eight archived NEOCs and seven normal ovaries. The RNA samples with RNA integrity number >2.0, purity >1.7 and cycle count value <28 cycles were hybridized to the Illumina Whole-Genome DASL assay (cDNA-mediated annealing, selection, extension, and ligation). We analyzed the results using the GeneSpring GX11.0 and FlexArray software to determine the differentially expressed genes. Microarray results were validated using an immunohistochemistry method. Statistical analysis identified 804 differentially expressed genes with 443 and 361 genes as overexpressed and underexpressed in cancer, respectively. Consistent findings were documented for the overexpression of eukaryotic translation elongation factor 1 alpha 1, E2F transcription factor 2, and fibroblast growth factor receptor 3, except for the down-regulated gene, early growth response 1 (EGR1). The immunopositivity staining for EGR1 was found in the majority of cancer tissues.

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This finding suggested that the mRNA level of a transcript did not always match with the protein expression in tissues. The current gene profile can be the platform for further exploration of the molecular mechanism of NEOC.


## Introduction

Ovarian cancer is among the most common gynecologic cancer and it is also a leading cause of death in gynecologic malignancies. In Malaysia, ovarian cancer accounts as the most common cancer in women after the breast, cervix uteri and colon. Around 1627 women in Malaysia were diagnosed with ovarian cancer between years 2003–2005 alone. Ovarian cancer is often referred as the 'silent killer' due to the absence of specific symptoms and lack of effective screening program.

Ovarian cancer is grouped into two types based on the origin of the cells, which either arises from the surface of epithelium or other cells of the ovary. The nonepithelial ovarian cancer (NEOC) is the less common subtype, which only represents about 10–15% of overall ovarian cancers [1]. NEOC has better prognosis than the epithelial ovarian tumor, but the recurrence rate is high which leads to the high mortality [2].

The etiology and molecular changes of NEOC are still poorly understood. NEOC occurs in all ages including children, young women, and postmenopausal women; therefore, it is imperative that these tumors are managed with accurate diagnosis, staging, and treatment. The tumor markers that are widely used to diagnose NEOC are CA-125, alpha-fetoprotein, beta human chorionic gonadotropin, inhibin, and estradiol [3–5]. However, the existing tumor markers are not specific and sensitive enough for the diagnosis of NEOC.

Therefore, there is a need to improve our understanding on the molecular biology of NEOC. The common goal of this study was to identify the potentially commonly expressed genes that have roles in the biology of NEOC. In this report, we used an oligonucleotide microarray to identify and compare differential gene expression patterns in formalin fixed paraffin embedded (FFPE) NEOC with normal ovaries sampled from a tertiary hospital in Malaysia.

## Methods

### Clinical samples

This study was conducted following institutional ethical clearance (Ref. UKM 1.5.3.5/244/SPP3). The sample size was computed using the online calculator provided by the University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA. The sample consists of eight NEOC and seven normal ovaries. The archived NEOC specimens were acquired from patients that underwent total abdominal hysterectomy-bilateral salpingo-oophorectomy (TAHBSO) for NEOC at the Universiti Kebangsaan Malaysia Medical Centre, Cheras, Kuala Lumpur between the years 2002 and 2008 and patients had a mean age of 35.1 $\pm$ 16.1 years. NEOC specimens it has been classified into five different subtypes. Out of eight NEOC specimens, three are granulosa cell tumors, two are mixed germ cell tumors, one is the yolk sac tumor, one is the immature teratoma, and one is the malignant mature teratoma. The normal ovarian tissues were obtained from uterine fibroid patients who underwent the TAHBSO with a mean age of 48.9 $\pm$ 3.0 years.

### RNA isolation and quality control of RNA

RNA was extracted from a pool of four to fie FFPE sections with 5-$\mu$m thickness using the High Pure RNA Paraffin Kit (Roche Diagnostics GmbH, Mannheim, Germany). RNA purities were measured using NanoDrop-1000 Spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). RNA integrity number was evaluated using the 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) with the RNA 6000 Nano kit following the manufacturer's protocols. A prequalification step was performed using quantitative polymerase chain reaction using primers product of RPL13a (accession number NM_012423.2) on Rotor Gene (Corbett Life Science) with SensiMix One-Step Kit (Quantace Ltd, London, United Kingdom).

### Microarray assay

The WG-DASL (cDNA-mediated Annealing, Selection, Extension, and Ligation) assay (Illumina, San Diego, CA, USA), which quantifies 24,526 transcripts, was performed following the manufacturer's protocol. This assay improves the capacity of the target set and retains the ability to profile partially degraded RNA samples [6,7].

### Statistical analysis

Microarray data analysis was performed using GeneSpring GX11.0.2 (Agilent Technologies, Santa Clara CA, USA) and FlexArray version 1.4.1 (Genome Quebec Innovation Centre, Montréal, Canada). Differential genes were identified using the unpaired *t* test, at $p < 0.005$ and two-fold changes. The gene set enrichment analysis (GSEA), gene ontology, and Pathway Studio were used to understand the descriptions of each gene product.

### Validation of gene expression differences

Due to the low RNA yield from the FFPE tissues, the immunohistochemistry (IHC) method was used as a validation tool. IHC is a practical and robust method for extending gene expression data to common pathologic specimens with the advantage of being applicable to FFPE tissues. Antibodies that were used in the IHC included anti-eukaryotic translation elongation factor 1 alpha 2 [EEF1A2

(Proteintech Group Inc, Chicago, USA)], anti-E2F transcription factor 2 [E2F2 (Bioworld Technology, Minneapolis, USA)], anti-fibroblast growth factor receptor 3 [FGFR3 (Abcam, Massachusetts, USA)] and anti-early growth response 1 [EGR1 (Abcam, Massachusetts, USA)]. Antigen was retrieved prior to staining. Endogenous peroxidase activity was blocked using 3% $H_2O_2$ followed by primary antibody incubation for 30 minutes. The bound antibody was detected using the REAL Envision Detection System (Dako, Glostrup, Denmark) for a 30-minute incubation period. Color was developed with 3,3′ Diaminobenzidine (DAB) for 10 minutes and counterstained with hematoxylin.

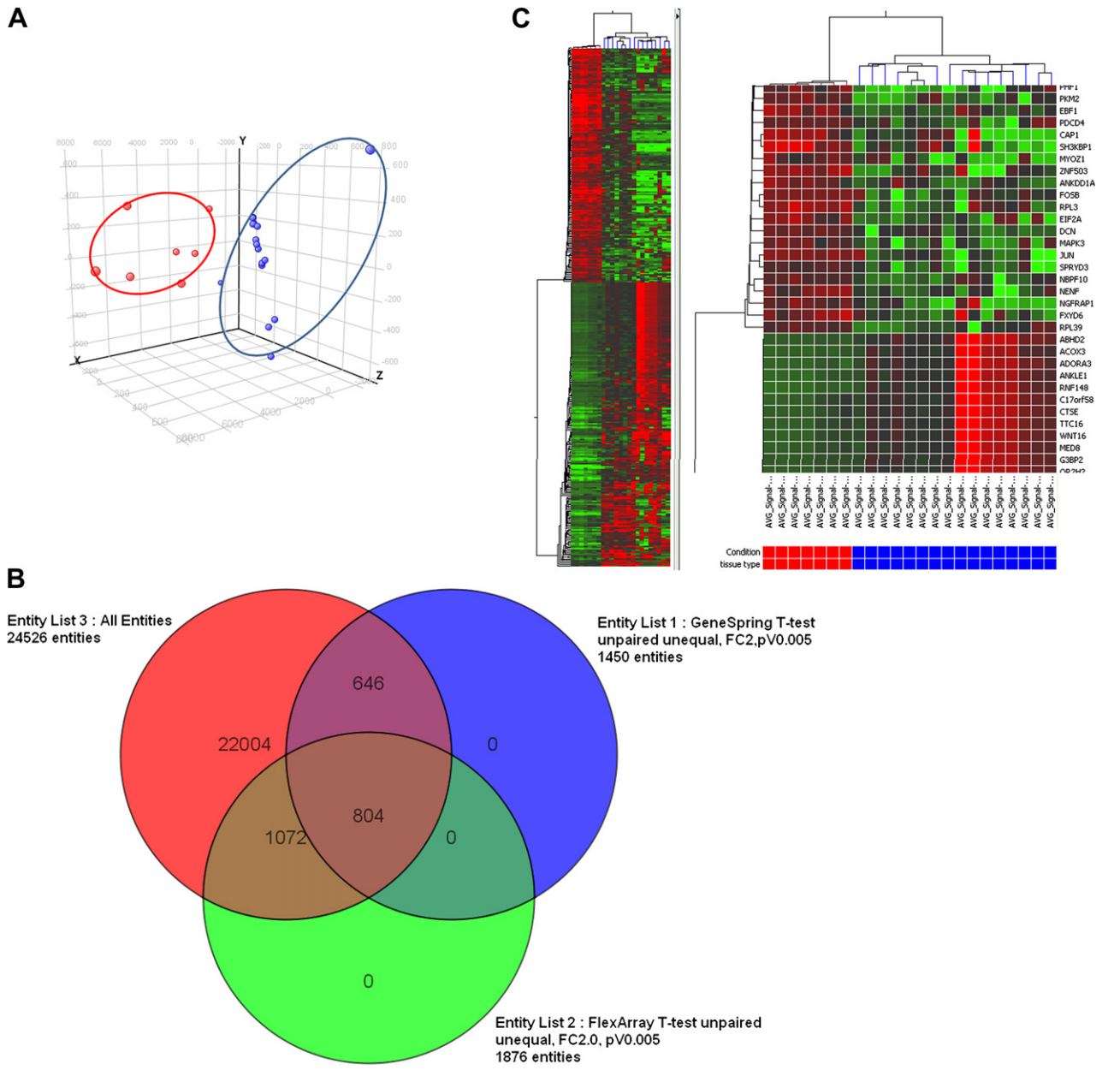


**Figure 1.** Microarray data analysis in 16 nonepithelial ovarian cancer and seven normal ovaries samples. (A) Principal component analysis clustered gene expression profiling of nonepithelial ovarian cancer and normal ovaries. All seven normal samples were clustered together and separated from the tumor group. The blue circle indicates the nonepithelial ovarian cancer and red circle indicates the normal ovaries; (B) Venn diagram showing 804 significant differentially expressed genes detected in both GeneSpring and FlexArray data analysis; (C) hierarchical clustering of 23 ovaries samples graphically displays the intensity of the gene expression for each gene. Samples were clustered based on 804 significant differentially expressed in nonepithelial ovarian cancers versus normal ovaries of at least two-fold ($p < 0.005$). The color of each square box represents the ratio of gene expression. Red indicates genes were up-regulated above the median; green, down-regulated; and black, equal to the median expression signal. The columns represent individual tissue samples; the rows represent individual genes.

**Table 1** Gene set enrichments analysis with q < 0.4 using the GeneSpring GX 11.0.2 software.

| Gene Set | *q* value (T vs N) | ES (T vs N) | Gene symbol | Entrez Gene | Gene description | Fold change | *p* value |
|---|---|---|---|---|---|---|---|
| MAHADEVAN_IMATINIB_ RESISTANCE_UP | 0.24 | 0.6430 | GREM1 | 26585 | Gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis), (GREM1) | +4.79 | 1.60E-05 |
| | | | NNMT | 4837 | Nicotinamide N-methyltransferase (NNMT) | +3.04 | 4.80E-04 |
| | | | SCN3A | 6328 | Sodium channel, voltage-gated, type III, alpha subunit (SCN3A), transcript variant 1 | +3.58 | 4.97E-05 |
| | | | SPANXB1 | 728695 | SPANX family, member B1 (SPANXB1) | +3.84 | 4.44E-04 |
| BIOCARTA_G2_PATHWAY | 0.28 | 0.5555 | CCNB1 | 891 | Cyclin B1 (CCNB1) | +9.07 | 1.67E-09 |
| | | | CDC25C | 995 | Cell division cycle 25 homolog C (*S pombe*), (CDC25C), transcript variant 2 | +10.92 | 4.26E-06 |
| | | | MDM2 | 4193 | Mdm2 p53 binding protein homolog (mouse), (MDM2), transcript variant MDM2b | +8.06 | 2.90E-04 |
| KAUFFMANN_MELANOMA_ RELAPSE_UP | 0.32 | 0.4678 | CDC45L | 8318 | CDC45 cell division cycle 45-like (*S cerevisiae*), (CDC45L) | +3.85 | 4.02E-04 |
| | | | FANCD2 | 2177 | Fanconi anemia, complementation group D2 (FANCD2), transcript variant 1 | +7.97 | 3.76E-06 |
| | | | PTTG1 | 9232 | Pituitary tumor-transforming 1 (PTTG1) | +10.72 | 2.47E-06 |
| | | | PTTG2 | 10744 | Pituitary tumor-transforming 2 (PTTG2) | +3.17 | 3.73E-05 |
| | | | RAD51 | 5888 | RAD51 homolog (RecA homolog, *E coli*) (S. cerevisiae), transcript variant 2 | +3.39 | 7.55E-05 |
| NADERI_BREAST_CANCER_ PROGNOSIS_DN | 0.35 | −0.6113 | C1S | 716 | Complement component 1, s subcomponent (C1S), transcript variant 1 | −15.79 | 2.19E-10 |
| | | | DCN | 1634 | Decorin (DCN), transcript variant A1 | −8.45 | 1.86E-04 |
| | | | SMOC2 | 64094 | SPARC related modular calcium binding 2 (SMOC2) | −3.10 | 1.03E-04 |
| BIOCARTA_TEL_PATHWAY | 0.32 | −0.6338 | EGFR | 1956 | Epidermal growth factor receptor [erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian (EGFR)], transcript variant 1 | −10.83 | 1.80E-06 |

This table shows description, fold change and *p*-value of five-significant gene sets with 16 significant genes with two-fold and $p < 0.005$.

## Results

### Gene expression patterns in NEOC

The expression patterns of NEOC were analyzed based on seven normal and duplication of eight tumor ovaries. The principal component analysis as a quality control on arrays samples distinctly separated the two different groups of samples (Fig. 1A). The unpaired *t* test with a cutoff ratio of at least two-fold and $p < 0.005$ in NEOC samples was compared with normal ovaries (Fig. 1B). Out of these 804 genes, 443 genes were up-regulated and 361 genes were down-regulated. The hierarchical clustering was performed on significant genes to arrange genes and samples in groups based on the similarity of the gene expression (Fig. 1C).

From the significant genes, positive regulation of transcription from RNA polymerase II promoter was among the significant gene set involved in biologic process. Fourteen genes were found, including ABLIM1, CREBBP, EGR1, GRIN1, JUN, NFAT5, NODAL, NR4A1, NR4A3, NR5A1, PBX1, SOX15, ST5, and THRA. EGR1 was detected among the top 10 down-regulated genes, and it was chosen for validation assay.

GSEA is a computational method that helps to connect the gene expression profile with their biologic categories [8]. The GSEA with $q < 0.4$ showed five significant gene sets with 16 genes were overlapped with the list of 804 significant genes [$p < 0.005$ (Table 1)]. The MAHADEVAN_IMATINIB_RESISTANCE_UP was the most enriched gene set, including GREM1, NNMT, SCN3A, and SPANXB.

The Pathway Studio analysis showed direct interaction of significant genes involved in cancers and ovarian cancer. The E2F2 and EGR1 were found to be associated with the MDM2 gene in BIOCARTA_G2_PATHWAY. The EEF1A2, E2F2, and EGR1 showed to have direct interaction with ovarian cancer, while FGFR3 has a direct interaction with other cancers. We chose EGR1, E2F2, EEF1A2, and FGFR3 for further validation assay.

### IHC

The results for IHC were based on 16 normal and 16 NEOC specimens. Positive immunostaining was detected in NEOC samples for EEF1A2, E2F2, and FGFR3 (Fig. 2). EEF1A2 immunostaining was parallel with mRNA expression in the microarray data. High expression of EEF1A2 protein was detected in the cytoplasm of granulosa cell tumors and mixed germ cell tumors of dysgerminoma tissues (Fig. 2B and C). Immunostaining for E2F2 in NEOC was also consistent with microarray data, showing a moderate to strong cytoplasmic and focally nuclear staining (Fig. 2E, F). The high FGFR3 mRNA expression in NEOC was consistently reflected in the moderate to strong staining of

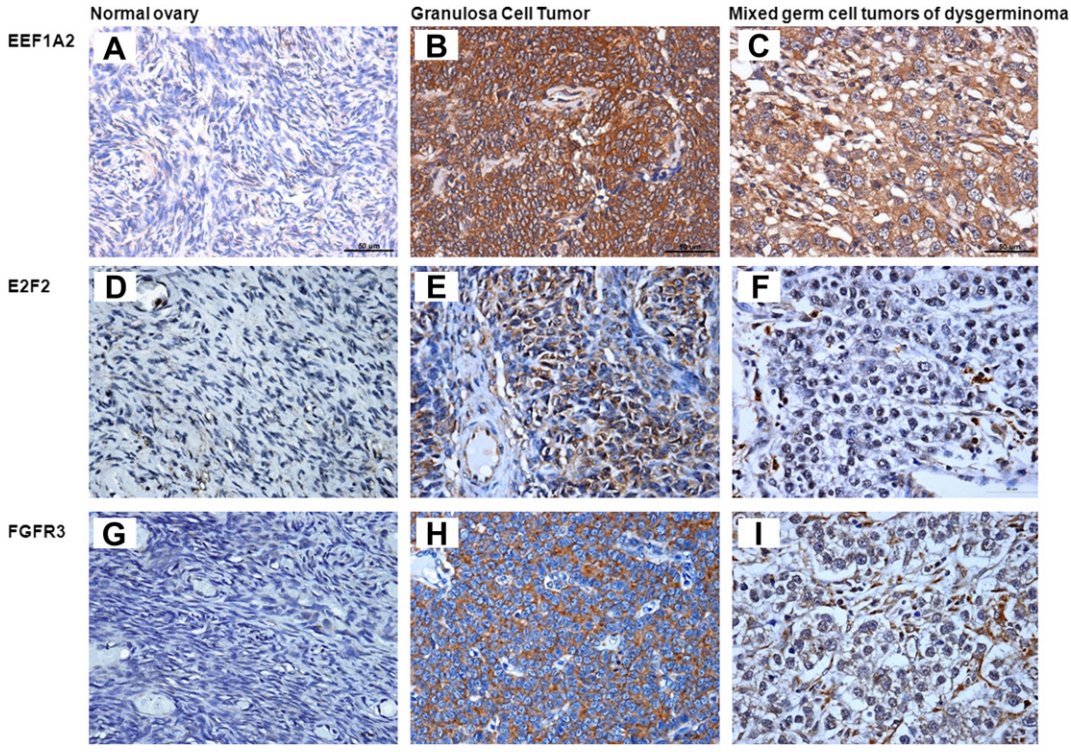


**Figure 2.** Immunohistochemistry of three differentially gene products in nonepithelial ovarian tissue samples. (A, D, and G) Negative immunostaining of EEF1A2, E2F2, and FGFR3 on a normal ovary; (B and C) positive immunostaining of EEF1A2 with strong cytoplasmic staining in granulosa cell tumors and mixed germ cell tumors of dysgerminoma tissues; (E and F) immunostaining for E2F2 shows cytoplasmic positivity and a few positive nuclei in both NEOC subtypes; (H and I) positive immunostaining for FGFR3 is predominantly in the membrane protein and stromal signals. Original magnification ×400. E2F2 = E2F transcription factor 2; EEF1A2 = eukaryotic translation elongation factor 1 alpha 2; FBFR3 = fibroblast growth factor receptor 3; NEOC = nonepithelial ovarian cancer.

predominantly membranous and some of the cytoplasm (Fig. 2H and I). The validation experiment for EGR1 showed an opposite result. Our microarray data showed down-regulation of EGR1 in NEOC, but the IHC result exhibited strong expression in all normal ovaries (Fig. 3A and B) as well as in 13 of the 16 NEOC samples (Fig. 3C). Only three of the 16 NEOC samples gave negative expression of EGR1 (Fig. 3D). The summary of IHC results on NEOC subtypes shown in Table 2. The validation data were shown in Table 3.

**Table 2** Summary of IHC results.

| | EEF1A2 | E2F2 | FGFR3 | EGR1 |
|---|---|---|---|---|
| NEOC subtype | | | | |
| Granulosa cell tumor | 6/6 | 6/6 | 6/6 | 4/6 |
| Juvenile granulosa cell tumor | 1/1 | 1/1 | 1/1 | 1/1 |
| Mixed germ cell tumor | 2/2 | 2/2 | 2/2 | 2/2 |
| Yolk sac tumor | 2/2 | 2/2 | 2/2 | 2/2 |
| Immature teratoma | 1/1 | 1/1 | 1/1 | 1/1 |
| Malignant mature teratoma | 1/1 | 1/1 | 1/1 | 0/1 |
| Dysgerminoma | 2/2 | 2/2 | 2/2 | 2/2 |
| Thecoma | 1/1 | 1/1 | 1/1 | 1/1 |
| | 16/16 (100%) | 16/16 (100%) | 16/16 (100%) | 13/16 (81%) |

E2F2 = E2F transcription factor 2; EEF1A2 = eukaryotic translation elongation factor 1 alpha 2; EGR1 = early growth response 1; FBFR3 = fibroblast growth factor receptor 3; IHC = immunohistochemistry; NEOC = nonepithelial ovarian cancer.

## Discussion

Several published studies have reported the use of microarray analysis in gene expression on ovarian neoplasm but those are limited to the epithelial ovarian cancer subtypes [9–11]. In the current study, we focused on analyzing the gene expression of NEOC. The availability of kits to extract RNA from the archived tissues for genome wide expression analysis has provided a great opportunity to characterize the molecular profiles of this tumor despite its rarity and limited availability of fresh tissues.

To assess the differential gene expression in NEOC, we have performed a microarray study using WG-DASL assay.

The NEOC is a heterogenous group of malignancies at both the histologic and genetic levels. These are all uncommon cancers. They are rare, and the literature is sparse with regard to the diagnosis and prognosis of this cancer. The molecular pathways involved in the pathogenesis of NEOC are still largely unknown. A profile of the common significant genes from different NEOC subtypes has been identified. There were 804 genes differentially expressed with at least two-fold change ($p < 0.005$). GSEA showed the connection between the significant genes that

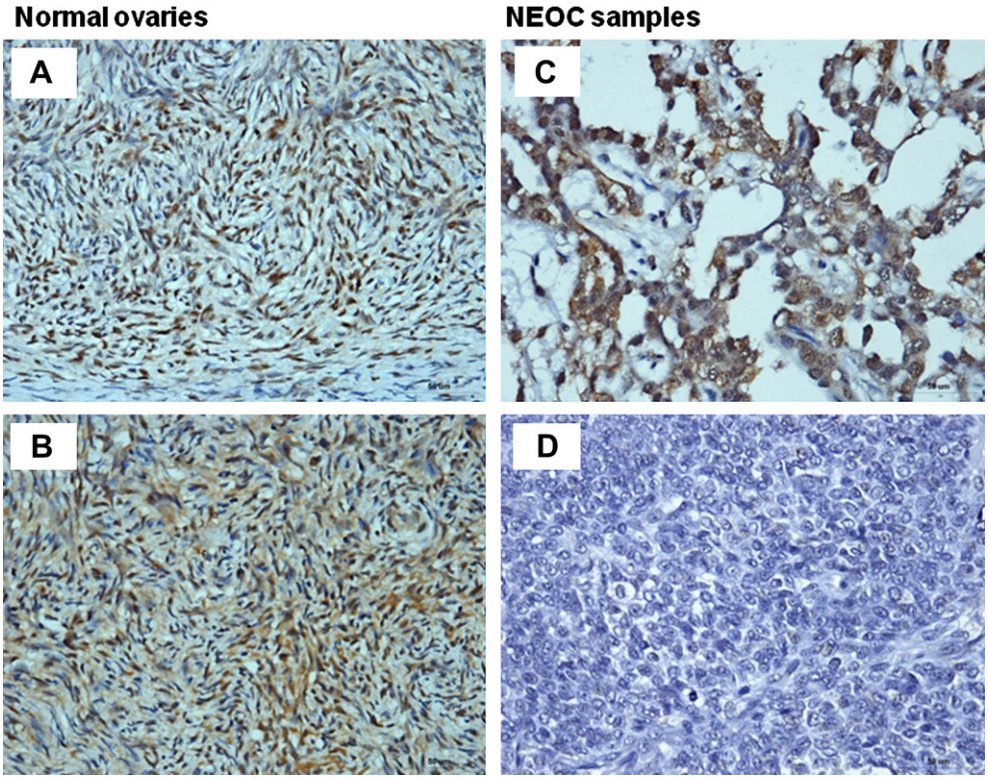


**Figure 3.** Immunohistochemistry of EGR1 for the validation of microarray data. (A, B) Positive immunostaining of EGR1 with nucleus and cytoplasmic in all the normal ovaries; (C) positive immunostaining of EGR1 is seen in the majority of the NEOC samples; (D) negative immunostaining of EGR1 in the minority of NEOC samples. Original magnification ×400. EGR1 = early growth response 1; NEOC = nonepithelial ovarian cancer.

**Table 3** Expression of genes and protein in NEOC by microarray and immunohistochemistry analysis.

| Gene/protein | Microarray data | Protein expression positive/ NEOC specimens[a] by IHC | Staining pattern |
|---|---|---|---|
| EEF1A2 | Up-regulated 41.14-fold | 16/16 | Cytoplasmic |
| E2F2 | Up-regulated 34.53-fold | 16/16 | Cytoplasmic and nuclei |
| FGFR3 | Up-regulated 20.93-fold | 16/16 | Cell membrane |
| EGR1 | Down-regulated 29.81-fold | 13/16 | Cytoplasmic and nuclei |

This table shows the expression level of four significant genes in NEOC compared with normal ovaries.
IHC = immunohistochemistry; NEOC = nonepithelial ovarian cancer.
[a] NEOC specimens ($n = 16$) with confirmed diagnosis of NEOC. Cases with at least minimal focal staining were defined to be positive.

share the common biologic functions. Among the significant pathways involved was the BIOCARTA_G2_PATHWAY, and MDM2 was identified as one of the significant genes. This pathway involved in the cell cycle phases, where there is a period of rapid cell growth and synthesis of protein for mitosis [12]. The increased level of MDM2 could be a result of various mechanisms, including enhanced translation and the inactivation of apoptotic and cell cycle arrest function of the p53 tumor suppressor gene [12,13]. MDM2 has an ability to inhibit the activity of the p53 and commonly occurs in the genesis of many tumors.

EEF1A2 was found to be highly expressed in NEOC compared to normal ovaries. This gene normally has high expression in muscles, neurons, and the heart [14]. A previous study reported that EEF1A2 was identified as an important player in the progression of tumors including that of the breast, lung, and ovary [15]. The high expression of EEF1A2 has implied that this gene possibly acts as an oncogene, activates tumorigenesis, and regulates apoptosis [16]. The high expression of EEF1A2 was supported by previous studies in breast cancer [17], epithelial ovarian cancer [15,18], and the hepatocellular carcinoma cell line, JHH6 [19].

Several others cancer genes were also overexpressed in NEOC, notably E2F2. Our findings are in agreement with previous studies in ovarian cancer and ovarian cell lines [20–22]. E2F2 was consistently overexpressed in NEOC. E2F2, which belongs to the E2F family, plays an important role in controlling cell cycle and acts as a tumor suppressor protein [23,24]. The abnormal activity of E2F transcription factor is the key event in development of most human cancers and may alter the p16-cyclin D-Rb pathway [21,23]. Previous studies have revealed that the overexpression of E2F2 may cause an excess of proliferation-promoting stimuli to the cancer cells [20]. The up-regulation of E2F2 in ovarian cancer cell lines may contribute to the uncontrolled proliferation and may also be associated with the highly malignant and fast growing tumors. Positive staining of E2F2 has suggested the high possibility of this gene acting as an oncogene in NEOC.

FGFR3 belongs to the fibroblast growth factor family, which is involved in various biologic processes, including cell growth, motility, differentiation, and angiogenesis [25]. Recent reports showed that deregulation of FGFR3 might affect cell growth and proliferation [25]. The high expression of FGFR3 as identified by our microarray as well as the IHC is consistent with findings in other cancers such as prostate carcinoma, cervical cancer, and myeloma [26].

EGR1 is a transcription factor involved in various biologic functions such as regulation of proliferation, growth, apoptosis and angiogenesis [27]. It was reported to be associated with cancer progression. In our study, we did not observe a consistent result between the microarray and IHC. The EGR1 showed positive immunostaining in NEOC despite showing low expression in the microarray data. The positive finding from IHC is consistent with the previous studies on high expression of EGR1 in prostate cancer [28]. The inconsistency of this result might also be explained by the low correlation between mRNA expression of this gene and its protein expression [29]. This could be due to biologic factors such as the complexity of the mechanisms involved between transcription and translation.

In conclusion, the present study successfully examined the gene expression profile of NEOC from a highly degraded FFPE samples using the oligonucleotide microarray assay. A set of significant genes was found to be differentially expressed in NEOC. This is the first local data that will be an informative platform for the future studies on the NEOC. Multiple NEOC subtypes did not make the data homogenous. Therefore, further studies using larger number of samples for each NEOC subtypes may be necessary to address the roles of cancer related genes and their biologic functions involved in NEOC.

## Acknowledgment

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## References

[1] Sanusi FA, Carter P, Barton DP. Non-epithelial ovarian cancers. Obstet Gynaecol 2000;2:37–9.
[2] Fuller PJ, Chu S. Signalling pathways in the molecular pathogenesis of ovarian granulosa cell tumours. Trends Endocrinol Metab 2004;15:122–8.
[3] Guillem V, Poveda A. Germ cell tumours of the ovary. Clin Transl Oncol 2007;9:237–43.
[4] Dällenbach P, Bonnefoi H, Pelte MF, Vlastos G. Yolk sac tumours of the ovary: an update. Eur J Surg Oncol 2006;32:1063–75.
[5] Pectasides D, Pectasides E, Psyrri A. Granulosa cell tumor of the ovary. Cancer Treat Rev 2008;34:1–12.

[6] Fan JB, Gunderson KL, Bibikova M, Yeakley JM, Chen J, Wickham Garcia E, et al. Illumina universal bead arrays. Methods Enzymol 2006;410:57—73.
[7] Chien J, Fan JB, Bell DA, April C, Klotzle B, Ota T, et al. Analysis of gene expression in stage I serous tumors identifies critical pathways altered in ovarian cancer. Gynecol Oncol 2009;114:3—11.
[8] Subramaniana A, Tamayoa P, Moothaa VK, Mukherjeed S, Eberta BL, Gillettea MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. PNAS 2005;102:15545—50.
[9] Davidson B, Zhang Z, Kleinberg L, Li M, Flørenes VA, Wang T-L, et al. Gene expression signatures differentiate ovarian/peritoneal serous carcinoma from diffuse malignant peritoneal mesothelioma. Clin Cancer Res 2006;12:5944—50.
[10] Le Page C, Ouellet V, Madore J, Ren F, Hudson TJ, Tonin PN, et al. Gene expression profiling of primary cultures of ovarian epithelial cells identifies novel molecular classifiers of ovarian cancer. Br J Cancer 2006;94:436—45.
[11] Warrenfeltz S, Pavlik S, Datta S, Kraemer ET, Benigno B, McDonald JF. Gene expression profilling of epithelial ovarian tumours correlated with malignant potential. Mol Cancer 2004;3:1—17.
[12] Jeczen R, Skomra D, Cybulski M, Schneider-Stock R, Szewczuk W, Roessner A, et al. P53/MDM2 overexpression in metastatic endometrial cancer: correlation with clinicopathological features and patient outcome. Clin Exp Metastasis 2007;204:503—11.
[13] Iwakuma T, Lozano G. MDM2, an introduction. Mol Cancer Res 2003;1:993—1000.
[14] Cao H, Zhu Q, Huang J, Li B, Zhang S, Yao W, et al. Regulation and functional role of eEF1A2 in pancreatic carcinoma. Biochem Biophys Res Commun 2009;380:11—6.
[15] Tomlinson VA, Newbery HJ, Wray NR, Jackson J, Larionov A, Miller WR, et al. Translation elongation factor eEF1A2 is a potential oncoprotein that is overexpressed in two-thirds of breast tumours. BMC Cancer 2005;5:1—7.
[16] Lee JM. The role of protein elongation factor eEF1A2 in ovarian cancer. Reprod Biol Endocrinol 2003;1:1—5.
[17] Kulkarni G, Turbin DA, Amiri A, Jeganathan S, Andrade-Navarro MA, Wu TD, et al. Expression of protein elongation factor eEF1A2 predicts favorable outcome in breast cancer. Breast Cancer Res Treat 2007;102:31—41.
[18] Pinke DE, Kalloger SE, Francetic T, Huntsman DG, Lee JM. The prognostic significance of elongation factor eEF1A2 in ovarian cancer. Gynecologic Oncol 2008;108:561—8.
[19] Grassi G, Scaggiante B, Farra R, Dapas B, Agostini F, Baiz DB, et al. The expression levels of the translational factors eEF1A 1/2 correlate with cell growth but not apoptosis in hepatocellular carcinoma cell lines with different differentiation grade. Biochimie 2007;89:1544—52.
[20] Reimer D, Sadr S, Wiedemair A, Stadlmann S, Concin N, Hofstetter G, et al. Clinical relevance of E2F family members in ovarian cancer—an evaluation in a training set of 77 patients. Clin Cancer Res 2007;13:144—51.
[21] Reimer D, Sadr S, Wiedemair A, Goebel G, Concin N, Hofstetter G, et al. Expression of the E2F family of transcription factors and its clinical relevance in ovarian cancer. Ann N Y Acad Sci 2006;1091:270—81.
[22] Reimer D, Sadr S, Wiedemair A, Concin N, Hofstetter G, Marth C, et al. Heterogeneous cross-talk of E2F family members is crucially involved in growth modulatory effects of interferon-gamma and EGF. Cancer Biol Ther 2006;5:771—6.
[23] Johnson DG, Schneider-Broussard R. Role of E2F in cell cycle control and cancer. Frontier Biosci 1998;3:447—58.
[24] DeGregori J. The genetics of the E2F family of transcription fators: shared functions and unique roles. Biochimica et Biophysica Acta 2002;1602:131—50.
[25] Sturla L-M, Merrick A, Burchill S. FGFR3IIIS: a novel soluble FGFR3 spliced variant that modulates growth is frequently expressed in tumour cells. Br J Cancer 2003;89:1276—84.
[26] Gowardhan B, Douglas D, Mathers M, McKie A, McCracken S, Robson C, et al. Evaluation of the fibroblast growth factor system as a potential target for therapy in human prostate cancer. Br J Cancer 2005;92:320—7.
[27] Weisz L, Zalcenstein A, Stambolsky P, Cohen Y, Goldfinger N, Oren M, et al. Transactivation of the EGR1 gene contributes to mutant p53 gain of function. Cancer Res 2004;64:8318—27.
[28] Krones-Herzig A, Adamson E, Mercola D. Early growth response 1 protein, an upstream gatekeeper of the p53 tumor suppressor, controls replicative senescence. PNAS 2003;100:3233—8.
[29] Pascal LE, True LD, Campbell DS, Deutsch EW, Risk M, Coleman IM, et al. Correlation of mRNA and protein levels: cell type-specific gene expression of cluster designation antigens in the prostate. BMC Genomics 2008;9:1—13.