Systems Biology in Reproductive Medicine

ISSN: 1939-6368 (Print) 1939-6376 (Online) Journal homepage: https://www.tandfonline.com/loi/iaan20

Molecular signatures of ovarian diseases: Insights from network medicine perspective

Medi Kori, Esra Gov & Kazim Yalcin Arga

To cite this article: Medi Kori, Esra Gov & Kazim Yalcin Arga (2016) Molecular signatures of ovarian diseases: Insights from network medicine perspective, Systems Biology in Reproductive Medicine, 62:4, 266-282, DOI: 10.1080/19396368.2016.1197982

To link to this article: https://doi.org/10.1080/19396368.2016.1197982

SYSTEMS BIOLOGY IN REPRODUCTIVE MEDICINE
2016, VOL. 62, NO. 4, 266–282
http://dx.doi.org/10.1080/19396368.2016.1197982

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Molecular signatures of ovarian diseases: Insights from network medicine perspective

Medi Kori, Esra Gov, and Kazim Yalcin Arga

Department of Bioengineering, Marmara University, Istanbul, Turkey

ABSTRACT

Dysfunctions and disorders in the ovary lead to a host of diseases including ovarian cancer, ovarian endometriosis, and polycystic ovarian syndrome (PCOS). Understanding the molecular mechanisms behind ovarian diseases is a great challenge. In the present study, we performed a meta-analysis of transcriptome data for ovarian cancer, ovarian endometriosis, and PCOS, and integrated the information gained from statistical analysis with genome-scale biological networks (protein-protein interaction, transcriptional regulatory, and metabolic). Comparative and integrative analyses yielded reporter biomolecules (genes, proteins, metabolites, transcription factors, and micro-RNAs), and unique or common signatures at protein, metabolism, and transcription regulation levels, which might be beneficial to uncovering the underlying biological mechanisms behind the diseases. These signatures were mostly associated with formation or initiation of cancer development, and pointed out the potential tendency of PCOS and endometriosis to tumorigenesis. Molecules and pathways related to MAPK signaling, cell cycle, and apoptosis were the mutual determinants in the pathogenesis of all three diseases. To our knowledge, this is the first report that screens these diseases from a network medicine perspective. This study provides signatures which could be considered as potential therapeutic targets and/or as medical prognostic biomarkers in further experimental and clinical studies.

Abbreviations DAVID: Database for Annotation, Visualization and Integrated Discovery; DEGs: differentially expressed genes; GEO: Gene Expression Omnibus; KEGG: Kyoto Encyclopedia of Genes and Genomes; LIMMA: Linear Models for Microarray Data; MBRole: Metabolite Biological Role; miRNA: micro-RNA; PCOS: polycystic ovarian syndrome; PPI: protein-protein interaction; RMA: Robust Multi-Array Average; TF: transcription factor

ARTICLE HISTORY
Received 14 January 2016
Revised 29 April 2016
Accepted 2 May 2016

KEYWORDS
Ovarian cancer; ovarian endometriosis; polycystic ovarian syndrome; reporter biomolecules; therapeutic targets

Introduction

Formation of mature oocytes and production of steroid hormones that are relevant to oogenesis and folliculogenesis occur in the ovary, and dysfunctions lead to various clinical or subclinical diseases, including polycystic ovarian syndrome (PCOS), ovarian cancer, premature ovarian failure [Richards and Pangas 2010], ovarian endometriosis [Aviel-Ronen et al. 2014], and ovarian cysts [Nahum et al. 2015]. Ovarian cancer, which can metastasize to extra-abdominal regions, is the second most encountered gynecologic cancer worldwide [Didžiapetrienė et al. 2014; Prat 2014].

Endometriosis is defined by the presence of uterine endometrial tissue in extra-uterine sites, and is observed in several abdominal regions, including ovary, peritoneum, retro-cervical area, retro-vaginal septum, rectum, bladder, appendix, and uterus [Simoens et al. 2007; Bellelis et al. 2011]; however, the ovary is the most common region for endometriosis [Aviel-Ronen et al. 2014]. Approximately 10% of the reproductive aged women are affected by endometriosis [Signorilea and Baldi 2015].

PCOS is another complicated, multi-factorial disorder that affects reproductive aged women [Sørensen et al. 2014]. PCOS is defined with hyperandrogenism, hirsutism and/or hyperandrogenemia, ovarian dysfunction, oligoanovulation and/or polycystic ovaries with excluded androgen excess or associated diseases [Azziz et al. 2009]. Its prevalence is reported as 6 to 20% in reproductive aged women [Johansson and Stener-Victorin 2013].

The etiological relationship between ovarian cancer and endometriosis has been studied for almost 90 years. Sampson [1925] described the association between endometriosis and ovarian cancer for the first time in 1925, and since then the studies have moved towards

CONTACT Kazim Yalcin Arga kazim.arga@marmara.edu.tr Department of Bioengineering, Faculty of Engineering, Marmara University, Building D, Office: 404, 34722 Kadikoy, Istanbul, Turkey.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/iaan.

understanding the mechanism. Though the relationship between endometriosis and ovarian cancer has been studied [Sayasneh et al. 2011], the molecular mechanistic basis, if any, for this connection are not yet certain. It was proposed that malignant transformation of endometriosis to ovarian cancer is multi-factorial, including genetic and hormonal factors [Pavone and Lyttle 2015]. Considering the observation that the risk of developing ovarian cancer is increased in women with PCOS [Chittenden et al. 2009], a relationship between these two diseases is also highly probable.

The availability of high-throughput functional genomics (i.e., transcriptomics, proteomics, and metabolomics) data will accelerate the understanding of the molecular mechanisms that underly ovarian diseases. Ovarian tissue specific diseases have been analyzed at the transcriptome level extensively. Wood et al. [2005] analyzed gene expression profiles in PCOS and identified that TRB3, a putative protein kinase induced by the transcription factor NF-kappaB, was significantly down-regulated whereas cAMP-GEFII, a cAMP sensor, was up-regulated at the transcript level. Furthermore, these altered genes were associated with basal and insulin-induced phosphorylation of protein kinase B (Akt/PKB). Moreno et al. [2007] used microarrays, and proposed that cellular proliferation, cell cycle DNA damage, and apoptosis were up-regulated in ovarian cancer. In the same year, Hever et al. [2007] conducted a transcriptome study in ovarian endometriosis. They reported that the differentially expressed genes were generally up-regulated, and lymphocyte stimulator (BLyS) protein was significantly over-expressed in ovarian endometriosis. Bowen et al. [2009] identified alterations in expression levels of gene products functioning in several signaling pathways including Wnt, Notch, TGFB/BMP, Hedgehog, and canonical cell cycle in ovarian cancer. Moreover, in another study that was carried out by Kenigsberg et al. [2009] differential expression of several members of Wnt/β-catenin and MAP-signaling pathways were reported in PCOS.

The corresponding metabolome has also been examined in recent years. For example, the eup-regulation of several fatty acid metabolites was revealed when metastatic ovarian tumors were compared with healthy tissues [Fong et al. 2011]. Analyzing plasma samples, the association of ovarian endometriosis with eight lipid metabolites were also identified [Vouk et al. 2012]. In another study, Atiomo and Daykin [2012] analyzed metabolites in plasma as a function of their differential levels in PCOS, and reported that several metabolites including citrulline, lipid, ornithine, proline, and acetone were down-regulated, and amino acids including arginine, glutamate, and organic acids such as citrate were significantly up-regulated.

More recently, data from studies focusing on transcriptional regulatory elements (i.e., transcription factors and microRNAs) have now become available. Llauradó and coworkers [2012] reported up-regulation of ETV5, which regulates cell adhesion in ovarian malignant cells, in ovarian cancer. Chang et al. [2013b] investigated the role of the transcription factor OCT4 in endometriosis, and found that it was over-expressed in endometriosis affected tissues. A few studies intended to identify differentially expressed miRNAs included Iorio et al. [2007] who compared miRNA profiles in ovarian cancer, and reported differential expression of 29 miRNAs. Similarly other studies reported differential expression of e.g., hsa-miR-200a, hsa-miR-200b, hsa-miR-200c, hsa-miR-182, hsa-miR-202, hsa-miR-483-5p, and hsa-miR-629, in ovarian endometriosis [Filigheddu et al. 2010; Laudanski et al. 2013]. Very recently, up-regulation of miR-93 in PCOS was also reported [Jiang et al. 2015].

Systems biology based network medicine is the application of network science towards identifying human diseases [Barabasi et al. 2011] utilizing genome-scale biological networks, high-throughput experimental datasets, and topological analysis methods. Understanding the molecular mechanisms behind ovarian diseases is a great challenge that requires the integration of biological information from different levels that is well suited to high-throughput functional genomics data and network-based approaches. Despite several experimental efforts, findings are generally inadequate to expose the central biological mechanisms of the pathologies that can map the interconnectivities between the diseases. Therefore, in the present study, we analyzed datasets from an integrated and comparative perspective (Figure 1). Using statistical analyses of the reported transcriptome datasets, we identified differentially expressed genes. For each dataset, protein-protein interaction networks were reconstructed and topological analyses were performed. Integration of the data with a genome-scale metabolic model and a comprehensive transcriptional regulatory network (transcription factor-miRNA-target gene) yielded prediction of reporter biomolecules (metabolites, proteins, transcription factors, and miRNAs) and biological signatures on central biological mechanisms of the pathologies.

Results

Transcriptomic signatures: Differentially expressed genes

In the present study, we employed and comparatively analyzed six gene expression datasets for ovarian cancer, ovarian endometriosis, and PCOS (Table 1). Statistical analyses were performed individually to identify DEGs

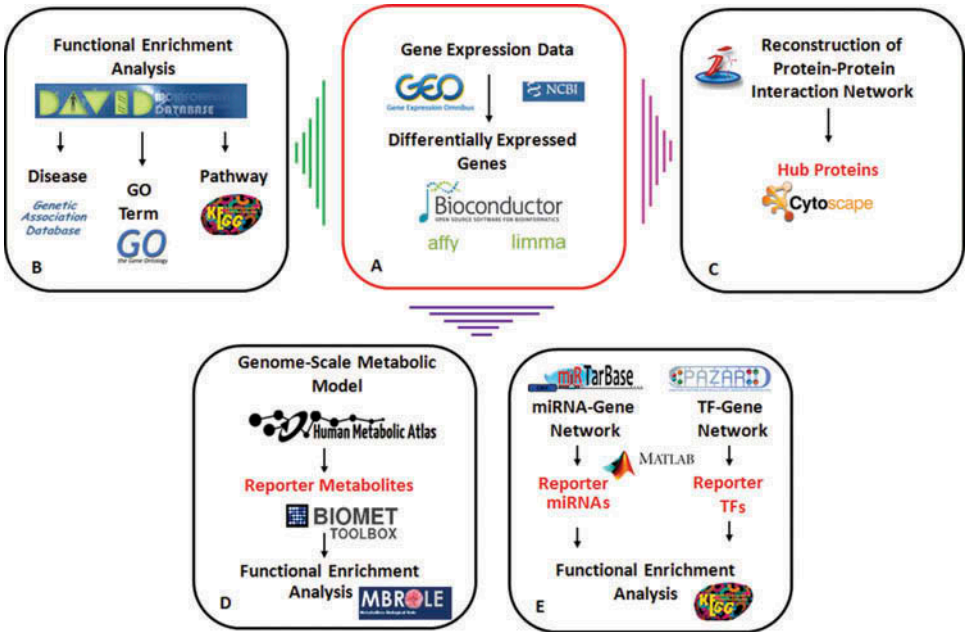


Figure 1. The multi-stage analysis methodology employed in the present study. (A) Gene expression datasets related to three ovarian tissue diseases were obtained from the Gene Expression Omnibus (GEO) database. Each dataset was statistically analyzed under Bioconductor platform to identify differentially expressed genes (DEGs). (B) Functional enrichment analyses of DEGs were performed to identify significantly enriched pathways, Gene Ontology annotation terms, and diseases. (C) Protein-protein interaction networks were reconstructed around DEGs, and the topological analyses were performed via Cytoscape to identify hub proteins. (D) DEGs were integrated into genome-scale metabolic model via BIOMET Toolbox to identify reporter metabolites, and pathway enrichment analyses were performed through Metabolite Biological Role (MBRole) tool to understand the biological roles of reporter metabolites. (E) To identify reporter micro-RNA (miRNA) and transcription factors (TF), miRNA-target gene and TF-target gene interactions were downloaded from miRTarbase and PAZAR databases, respectively. DEGs were integrated with those networks using MATLAB and the statistically significant ($p<0.05$) miRNAs and TFs were considered as the reporter transcriptional regulatory elements. The target DEGs of reporter TFs and miRNAs were subjected to pathway enrichment analyses.

and their regulatory patterns (i.e., up- or down-regulation) (Figure 2A). Moreover, functional enrichment analyses were performed on down- and up-regulated DEG sets.

As summarized in Figure 2A, a total of 8,119 genes, 5,100 down- and 3,019 up-regulated, were detected as differentially expressed among ovarian and non-ovarian cancer samples. In ovarian endometriosis 2,982 DEGs significantly down-regulated and 2,794 DEGs were significantly up-regulated considering disease and control states. Amongst PCOS and healthy samples, 1,179 genes were down-regulated whereas 618 genes were up-regulated. The comparative analyses of DEGs indicated that there were 403 mutual genes between three diseases, but their regulatory patterns (down or up) were not the same in all. Accordingly, 31 down-regulated genes and six up-regulated genes were mutual among all diseases when the regulatory patterns were also taken into account (Figure 2B,C).

Table 1. Transcriptome datasets employed in the present study.

GEO Reference Series	Disease State	Sample Subsets	Array	Reference
GSE7463	Ovarian Cancer	9 Diseased (Pre-Treated Ovarian Adenocarcinomas), 10 Control (Pre-Treated Benign Ovarian Serous Cystadenomas)	Affymetrix Human Genome U95 Version 2	Moreno et al. 2007.
GSE14407	Ovarian Cancer	12 Diseased (Ovarian Cancer Epithelial Cells), 12 Control (Normal Ovarian Surface Epithelial)	Affymetrix Human Genome U133 Plus 2.0	Bowen et al. 2009
GSE7305	Endometriosis	10 Diseased (Ovarian Endometriosis Tissue), 10 Control (Normal Endometrium Tissue)	Affymetrix Human Genome U133 Plus 2.0	Hever et al. 2007
GSE1615a	PCOS	5 Diseased (Untreated, Theca cells), 4 Control (Untreated, Theca Cells)	Affymetrix Human Genome U133A	Wood et al. 2005
GSE1615b	PCOS	5 Diseased (Untreated, Theca Cells), 4 Control (Untreated, Theca Cells)	Affymetrix Human Genome U133B	Wood et al. 2005
GSE10946	PCOS	5 Diseased (Lean, Cumulus Cells), 6 Control (Lean, Cumulus Cells)	Affymetrix Human Genome U133 Plus 2.0	Kenigsberg et al. 2009

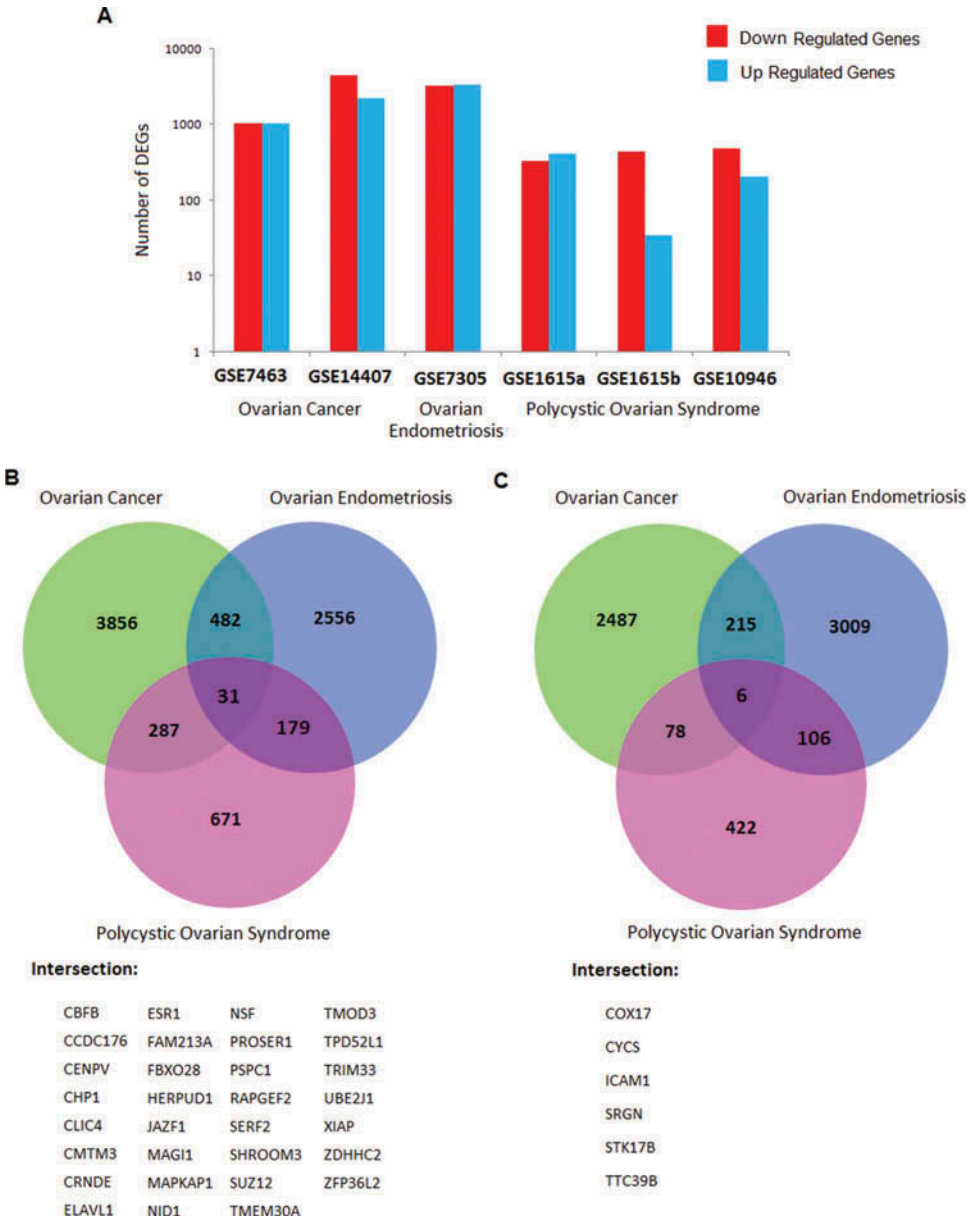


Figure 2. Differentially expressed genes (DEGs) and comparative analyses of mutual DEGs in ovarian tissue related diseases. (A) The distribution of (DEGs). For each dataset, DEGs which had statistically significant changes in their expression profiles among diseased and healthy samples were determined independently. Down-regulation and up-regulation of DEGs were represented by light and dark colors, respectively. (B) Venn diagram representing the comparison of down-regulated DEGs between the investigated diseases. The gene symbols representing the 31 mutual down-regulated DEGs were given below the diagram. (C) Venn diagram representing the comparison of up-regulated DEGs between the investigated diseases. The gene symbols representing the 6 mutual up-regulated DEGs were given below the diagram.

Most of the proteins encoded by mutual down-regulated genes were classified into four groups according to their functions or activities: (i) ELAVL1, HERPUD1, SHROOM3, SUZ12, TMOD3, and ZFP36L2 act as binding proteins, (ii) CHP1, CLIC4, MAGI1, MAPKAP1, NSF, RAPGEF2, TRIM33, UBE2J1, and XIAP are enzymes or enzyme inhibitors, (iii) ESR1 and NID1 serve as receptor, and (iv) JAZF1 and ZDHHC2 are zinc finger proteins. Among others: CENPV is a centromere protein, CMTM acts as a cytokine, FBXO28 is an F-box protein, PSPC1 is an mRNA splicing factor, FAM213A and CBFB are regulatory proteins, TMEM30A is a transmembrane protein, and TPD52L1 is a tumor associated protein. The remaining four proteins, CCDC176, CRNDE, PROSER1, and SERF2, are noted as uncharacterized. Among mutual up-regulated genes, COX17 is a carrier protein, CYCS acts as cytochrome c, ICAM1 is an

intercellular adhesion molecule, SRGN is a proteoglycan, STK17B is an enzyme (serine/threonine kinase), and TTC39B is uncharacterized.

The enrichment analyses based on situated DEGs for each disease indicated significant results for five pathways in all diseases: focal adhesion, adheres junction, ECM-receptor interaction, MAPK signaling pathway, and pathways in cancer (Figure 3A). On one hand, cell adhesion molecules, Fc gamma-R mediated phagocytosis, and systemic lupus erythematosus were up-regulated in both ovarian cancer and endometriosis. On the other hand, the WNT signaling pathway was down-regulated in both ovarian cancer and PCOS. The 403 DEGs common among the three diseases were statistically enriched with spliceosome, prostate cancer, Parkinson's disease, and small cell lung cancer in addition to these biological processes or molecular pathways.

Proteomic signatures: Hub proteins

Protein-protein interaction (PPI) networks were reconstructed around proteins encoded by DEGs via their physical interactions. For three diseases, PPI sub-networks were constructed individually around down and up-regulated DEGs. In topological analyses of the PPI sub-networks, degree (local-based) and betweenness centrality (global-based) metrics were simultaneously investigated to determine the highly connected proteins, i.e., hubs (Table 2), which might play significant roles in the disease progression.

Among hub proteins, SMAD2, SMAD3, AR, SIRT7, and PCNA were specific to ovarian cancer. SMAD3 and SMAD2 are receptor regulated SMAD (R-Smad) members and have crucial roles in the transforming growth factor-β (TGF-β) superfamily signaling pathway, which has been associated with cancer development [Shi and Massague 2003; Xue et al. 2014]. The androgen receptor (AR) is a widespread steroid receptor expressed in approximately 80% of ovarian cancer cases [Aragon-Ching 2014; Ligr et al. 2011], and expressed in mammary gland, ovary, uterus, fallopian tubes, and the vagina in mammals [Chang et al. 2013a]. SIRT7 belongs to the Sirtuin gene family, which may act as a tumor promoter or suppressor [McGlynn et al. 2015], and the role of SIRT7 as an oncogene has been previously identified in ovarian cancer [Wang et al. 2015]. Proliferating cell nuclear antigen (PCNA) plays a role in various cellular processes [Stoimenov and Helleday 2009] and is up-regulated in cancer cells [Moldovan et al. 2007].

Statistically most significant hub proteins for ovarian endometriosis were VHL, BRCA1, EGR1, FYN, and CTNNB1. VHL is a tumor suppressor, which is expressed in the ovary and endometrium tissues, and its inactivation may give rise to ovarian tumors [Lu et al. 2014]. BRCA1 mutations have a high incidence in women with breast cancer, and it has been proposed that reduced BRCA1 expression might play a substantial role in endometriosis pathophysiology [Govatati et al. 2014]. Similar to VHL, EGR1 also acts as a tumor suppressor, and may suppress glioma, lung, and breast cancer; however, its induction of

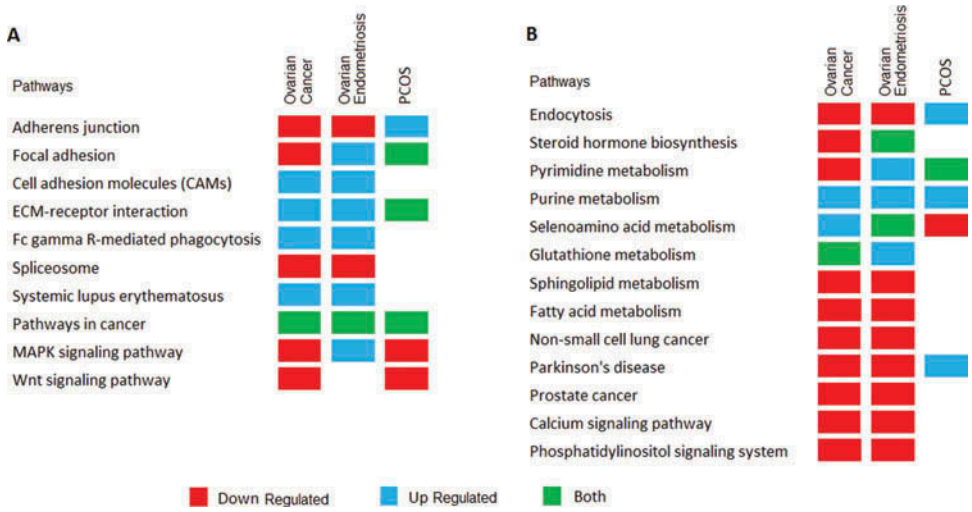


Figure 3. Significant enrichment results for signaling, regulatory, and metabolic pathways. (A) Pathway enrichment analysis of differentially expressed genes (DEGs) for the ovarian cancer, ovarian endometriosis, and polycystic ovarian syndrome (PCOS) via the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation tool based on the information on gene-reaction-pathway associations from Kyoto Encyclopedia of Genes and Genomes (KEGG) database for *Homo sapiens*. (B) Pathway enrichment analysis of reporter metabolites for the inspected diseases via the Metabolite Biological Role (MBRole) bioinformatics tool. Light grayscale represents down-regulation, whereas dark grayscale represents up-regulation. Pathways represented by intermediate grayscales include both down-regulated and up-regulated branches.

Table 2. Most significant reporter biomolecules ($p<0.05$) for reporter metabolites, proteins, TFs, and miRNAs.

Disease State	Gene Expression Profile	Hub Proteins	Reporter Metabolites	Reporter TFs	Reporter miRNAs
Ovarian Cancer	Down-Regulated	SMAD2, SMAD3, ESR1, AR, EGFR	5-hydroxyindoleacetaldehyde, 13-cis-retinal, 9-cis-retinoate, NAD, NADH	HIF1A, STAT1, STAT6, RUNX1, EPAS1, RB1, ESR1, TP53, SOX2, FOXA1, RBL2, TRIM28, EGR1, TAL1, PRDM14, ZNF263	miR-19b-3p, miR-130b-3p, miR-9-5p, miR-128-3p, miR-181a-5p, miR-122-5p, miR-148b-3p, miR-132-3p, miR-101-3p, miR-183-5p
	Up-Regulated	KIAA0101, YWHAZ, SIRT7, SUMO1, CDK1, PCNA, PPP1CA H2AFX,	Formate, 5,10-methylene-THF, succinate, nicotinamide ribonucleoside, xanthine	STAT1, EPAS1, FOS, STAT6, HIF1A, TFAP2C, RB1, RBL2, TP53, E2F1, RUNX1	miR-484, miR-24-3p, miR-125b-5p, miR-149-5p, miR-744-5p, miR-18a-3p, miR-877-3p, miR-222-3p, miR-331-3p, miR-423-3p, miR-93-3p
Ovarian Endometriosis	Down-Regulated	KIAA0101, TP53, YWHAZ, VHL, BRCA1, EGFR, CDK1, H2AFX, PPP1CA	Ceramide pool, 1,2-diacylglycerol-bile-PC pool, 1-lyso-2-arachidonoyl-phosphatidate, sphingosine-1-phosphate	RBL2, RB1, FOS, TFAP2C, STAT6, E2F4, ESR1, HIF1A, FOXA1, STAT1, EPAS1, TP53, ESR2	miR-34a-5p, miR-24-3p, miR-877-3p, miR-744-5p, miR-149-5p, let-7e-5p, miR-125b-5p, miR-18a-3p, miR-100-5p, let-7a-5p, miR-222-3p, miR-1260b, miR-196a-5p, miR-25-3p, miR-324-5p
	Up-Regulated	EGR1, FYN, CTNNB1, TGFBR1, HDAC5	Prostaglandin I2, selenomethionine, trimethylamine, choline, ethanolamine	STAT1, HIF1A, EPAS1, RUNX1, ESR1, STAT6, TP53, SOX2, BACH1, SPI1, FOXA1, TRIM28, RB1, EGR1, TAL1	miR-19b-3p, miR-130b-3p, miR-9-5p, miR-128-3p, miR-181a-5p, miR-122-5p, miR-148b-3p, miR-132-3p, miR-101-3p, miR-183-5p, miR-221-3p, miR-10a-5p, miR-146a-5p, miR-106b-5p
Polycystic Ovary Syndrome	Down-Regulated	MYC, TP53, ESR1, TGFBR1, MAPK1, HDAC5	Alanine, pyruvate, GD1a, GM1b, L-lysyl-tRNA(lys)	STAT6, FOS, RB1, AMPD3, CAND1.11, HIF1A, TP53, EGR1, ESR1, EPAS1, ZNF263, TFAP2C, DDIT3, RUNX1, STAT1, ETS1, NFYA, RBL2, FOXA1	miR-335-5p, miR-744-5p, miR-320a, miR-7-5p, miR-93-5p, miR-93-3p, let-7a-5p, let-7e-5p, miR-423-3p
	Up-Regulated	SUMO2, TP53, SUMO1, SRC, IKBKE	Ferricytochrome C, ubiquinol, (5Z,8Z,11Z)-eicosatrienoyl-CoA, activation-ppara	STAT6, RB1, HIF1A, RBL2, STAT1, E2F4 ESR1, ETS1, GATA3, TP53, FOXA1, EPAS1, ZNF263	miR-877-3p, miR-196a-5p, miR-197-3p, miR-100-5p, miR-15a-3p, miR-1236-3p, miR-1260b, miR-147b, miR-663b, miR-30c-5p, miR-15b-5p, miR-26a-5p

prostate cancer progression is noted [Kim et al. 2014]. Fyn expression is up- regulated in several cancers including melanoma, glioblastoma, prostate cancer, and squamous cell carcinoma of the head and neck [Saito et al. 2010]. Moreover, Fyn has been identified in mammalian oocytes [McGinnis et al. 2009]. CTNNB1 (β-catenin) is a core molecule of the WNT/β-catenin pathway. The dysregulation of this pathway was associated with many cancers including ovarian cancer, and CTNNB1 overexpression was observed in ovarian cancer [Bodnar et al. 2014].

MYC, MAPK1, SUMO2, SRC, and IKBE were the top highlighted hubs that were specific to PCOS. The MYC proto-oncogene, is activated or up-regulated in more than 50% of cancers. MYC generally behaves as a transcription factor and identified as one of the precursors of tumorigenesis [Gabay et al. 2014]. Dysregulation of multiple signaling pathways has been associated with PCOS and linked with androgen abundance [Ortega and Duleba 2013]. SUMO2 is a small ubiquitin-like modifier and takes a role in post-transcriptional regulation of several target proteins [Su and Li 2002]. Src is a protein kinase that has roles in tumor progression, invasion, and metastasis [Sen and Johnson 2011]. IKBKE is crucial for antiviral signaling pathway regulation, and is also defined as an oncogene in breast cancer [Hutti et al. 2009].

Additionally, according to comparative analysis of hub proteins, CDK1, EGFR, H2AFX, KIAA0101, PPP1CA, and YWHAZ were the mutual proteomic signatures for both ovarian cancer and endometriosis. With the exception of EGFR, their regulatory patterns were inversely correlated between two diseases (i.e., down-regulated in ovarian cancer, and up-regulated in ovarian endometriosis). Among those, CDK1, PPP1CA, and YWHAZ proteins have significant roles in oocyte meiosis. Considering ovarian endometriosis and PCOS, three hubs (HDAC5, TGFBR, and TP53) were prominent. Similarly, two proteins (ESR1 and SUMO1) were identified as mutual hubs for ovarian cancer and PCOS.

Metabolic signatures: Reporter metabolites

Reporter metabolite analyses were performed using situated DEGs, and down and up- regulated reporter metabolites were individually discovered for each pathology (Table 2). An aldehyde compound (i.e., 5-hydroxyindoleacetaldehyde), coenzymes NAD and NADH, and two retinoid derivatives (13-cis-retinal, and 9-cis-retinoate) were significantly down-regulated; whereas formate, 5,10-methylenetetrahydrofolate, succinate, nicotinamide ribonucleoside, and xanthine were

up-regulated in ovarian cancer. The metabolic profiling study by Ke and coworkers [2015] identified multiple specific metabolic biomarkers, including 5-hydroxyindoleacetaldehyde for epithelial ovarian carcinoma, and this was the first study that reports 5-hydroxyindoleacetaldehyde as an ovarian cancer biomarker. Retinoids are chemically related to vitamin A and have been studied as a substance for countless cancer treatments including ovarian cancer. It was proposed that a considerable intake of retinoid derivatives triggers a decline in ovarian cancer development [Whitworth et al. 2012]. Formate is the core metabolite of the folate-mediated one-carbon metabolic pathway which is significant for DNA synthesis, repair, and methylation. Assessment of the concentration of formate in folate and B12 absence, and folate absence was associated with increased cancer risk [Lamarre et al. 2013; Stevens et al. 2007]. Although no significant association between folate and ovarian cancer was reported, it was known that folate receptor alpha is up-regulated in ovarian cancer [Harris et al. 2012]. A core enzyme of folate metabolism, MTHFR, catalyzes the reduction of 5, 10-methylene THF to 5-methyl THF. Absence of folate has been associated with ovarian cancer, and the role of MTHFR in the carcinogenesis was reported [Zhang et al. 2012]. Succinate, an intermediate of the citric acid cycle, plays an essential role in ATP formation in mitochondria, and modifies proteins post-translationally. Succinate stabilizes the transcription factor HIF1A (hypoxia-inducible factor-1α) and activates macrophages in particular malignancies [Mills and O'Neill 2014]. Nicotinamide ribonucleoside (also called nicotinamide riboside) is a resource of niacin (vitamin B3) and precursor of NAD [Chi and Sauve 2013]. NAD is an essential component for energy and signaling pathways that regulate DNA repair, apoptosis, and cell cycle. Thereby NAD metabolism has been connected with cancer progression [Chiarugi et al. 2012]. Xanthine is the initial compound that shows the presence of abnormal purine profile. Despite the low xanthine serum level, it increases according to abnormal purine salvage pathways [Pundir and Devi 2014].

In ovarian endometriosis most significant under-expressed reporter metabolites were lysophosphatidic acid (1-lyso-2-arachidonoyl-phosphatidate), diacylglycerol (1,2-diacylglycerol-bile-PC pool), and two sphingolipid metabolites (i.e., ceramide, and sphingosine). On the contrary, a prostaglandin member (prostaglandin I2), selenomethionine, trimethylamine, and choline ethanolamine were s over-expressed reporter metabolites. The effect of lysophosphatidic acid on endometriosis and ovarian cancer was previously reported [Ye and Chun 2010]. Ceramide and sphingosine-1-phosphate are the two primary sphingolipids in various signaling pathways including apoptosis, stress responses, and intracellular trafficking. Curiously, they present opposing effects on cell survival. For example, ceramide leads to apoptosis whereas sphingosine-1-phosphate leads to cell survival [Gatt and Dagan 2012]. Prostaglandin I2 is a metabolite of the arachidonic acid pathway and high levels of prostaglandins were reported in peritoneal fluids of endometriosis patients [Cathcart et al. 2011; Meresman and Olivares 2012]. Selenomethionine has been identified as a probable agent that can be used in prostate cancers to inhibit, delay, or reverse carcinogenesis [Nyman et al. 2004]. Trimethylamine is an aliphatic amine, which has a potential to generate a highly toxic carcinogen compound N-nitrosodimethylamine, and cause aberrant neurological symptoms in end-stage renal patients [Bain et al. 2006]. Choline metabolites were reported as potential biomarkers for prostate cancer diagnosis [Awwad et al. 2012]. Ethanolamine is one of the substantial metabolites required for synthesis of two core phospholipids (phosphatidylcholine and phosphatidylethanolamine) which constitute more than 50% of the phospholipid content in eukaryotic membranes [Gibellini and Smith 2010].

Alanine, pyruvate, and several ganglioside molecules (GD1a, GM1b), were significantly down-regulated in PCOS. In contrast, ferricytochrome c ubiquinol, eicosatrienoyl coenzyme A, and 'activation-ppara' were the most significant over-expressed metabolites in PCOS. Modification of the glycolysis and alanine synthesis pathways were encountered in pre-tumor stages of cancers [Munoz-Pinedo et al. 2012]. Gangliosides, including glycosphingolipids, are abundant in central nervous system tissues. They are tumor-associated antigens, important cell-surface receptors, and they also attend in diverse biological processes (cell differentiation and growth) [Sun and Jiang. 2012]. Ubiquinol functions in proton/electron translocation in oxidative phosphorylation, and inhibition of apoptotic pathways [Sedlák et al. 2010]. Ubiquinol was proposed as a potential biomarker for oxidative stress and tissue energy requirements [Miles et al. 2005]. Furthermore, 'activation-ppara' reaction stimulates nuclear receptor protein encoded by PPARA gene.

Functional enrichment analyses of reporter metabolites demonstrated that several metabolic pathways (i.e., pyrimidine, purine, selenoamino acid metabolism) and Parkinson disease pathways were common in three diseases (Figure 3B). Non-small cell lung and prostate cancer disease pathways, spingolipid and fatty acid metabolic pathways, and calcium and phosohatidylinositol signaling pathways were highlighted down-regulated pathways for both ovarian cancer and endometriosis.

Regulatory signatures: Reporter TFs and miRNAs

Transcriptional and translational regulatory components, i.e., TFs and miRNAs, were also determined based on transcriptomic alterations in disease states. The results have been mapped onto the regulatory network, and highly significant TFs and miRNAs were defined as reporter transcriptional regulatory components (Table 2, Figure 4). Based on comparative analyses, disease specific and common transcriptional regulatory compounds were identified.

Reporter TFs, PRDM14, and E2F1 were highlighted in ovarian cancer, whereas ESR2, BACH1, and SPI1 were highlighted in endometriosis. In addition, AMPD3, CAND1.11, DDIT3, ETS1, NFYA, and GATA3 were reporter TFs specific to PCOS. Thitrteen TFs were determined as mutual transcriptional regulatory components for all three diseases (Figure 4A). We also screened reporter miRNAs (Figure 4B). In general, encountered reporter TFs and miRNAs were linked with cell cycle processes and cancer formation. For example, the E2F family (E2F1 and E2F4) have a core role in cell cycle control [Iwanaga et al. 2006]; EGR1 [Chen et al. 2010] and STAT family members STAT1 and STAT6 [Mitchell and John 2005] play a role in the regulation of cell growth, differentiation, and apoptosis. Differential DDIT3 expression was observed under cellular stress [Bento et al. 2009]; ETS1 behaves as a proto-oncoprotein [Dittmer 2003]. Similarly, miR-21, miR221, and miR222 family members function in oncogenesis [Hayes et al. 2014]. In comparison, RUNX1 [Goyama et al. 2013], TP53 [Beckerman and Prives 2010], BACH1 [Hira et al. 2007], miR-193b [Yang et al. 2014], and miR-124-3p [Li et al. 2014] act as tumor suppressors; and HIF1A [Anam et al. 2015], PRDM14 [Nishikawa et al. 2007], TRIM28 [Hatakeyama 2011], miR- 15b-5p [Yang et al. 2015], miR-30c-5p [Oksuz et al. 2015], and miR-16-5p [Zhang et al. 2015] have been associated with various cancers.

Comparative analyses revealed that 32 miRNAs were common transcriptional regulatory components for all three diseases. While there were mutual TFs and miRNAs between the three diseases, regulatory patterns of some of these components were inversely correlated.

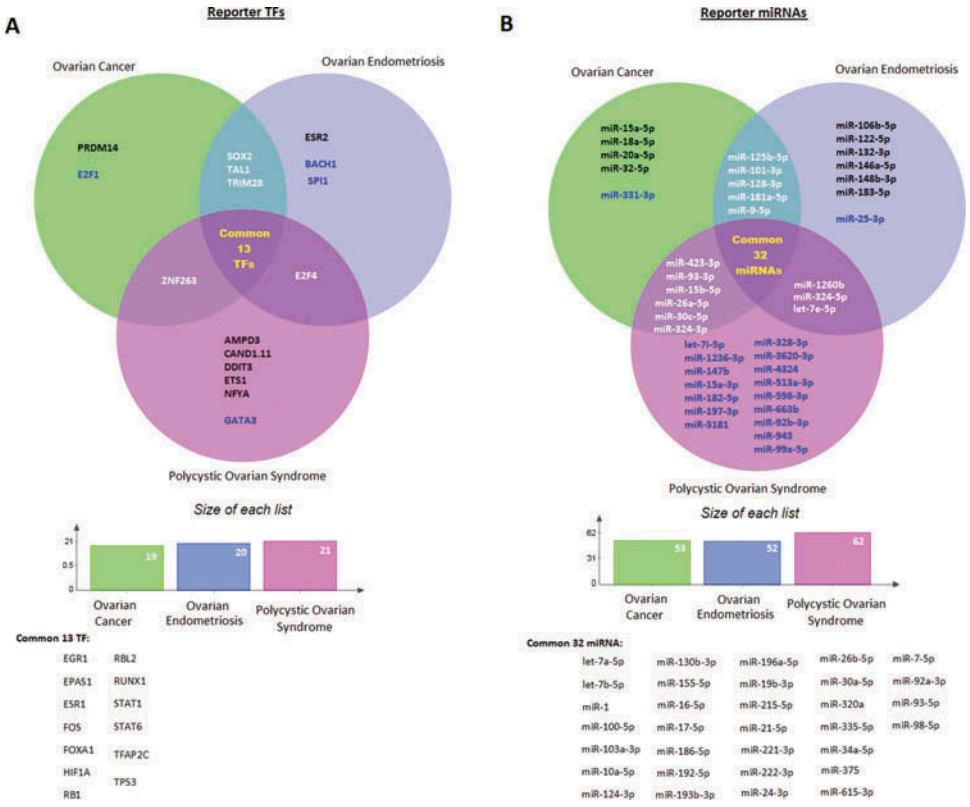


Figure 4. Venn diagrams representing the reporter transcription factors (TFs) and micro-RNAs (miRNAs). (A) Reporter TFs. (B) Reporter miRNAs. Venn diagrams were used to compare the reporter transcriptional regulatory elements among the three ovarian pathologies. The compounds determined via down-regulated differentially expressed genes (DEGs) were highlighted with black color; whereas the gray colored compounds were determined using up-regulated DEGs. The overlapping regulatory compounds were represented by white color. The gene symbols representing the mutual regulatory compounds (13 TFs and 32 miRNAs) are below the Venn diagrams.

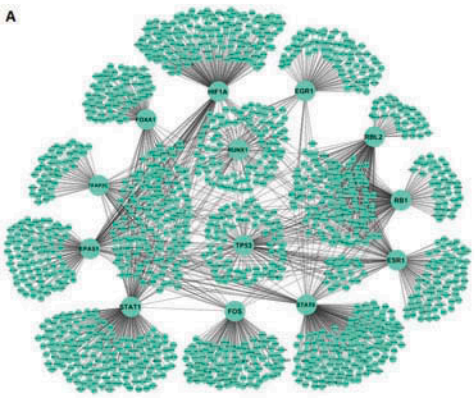
For example, the TFs: EGR1, TAL1, TRIM28, SOX2, and miRNAs: miR-101-3p, miR-128-3p, miR-181a-5p, miR-9-5p were down-regulated in ovarian cancer while they were up-regulated in ovarian endometriosis. On the contrary FOS, TFAB2C, and miR-125b-5p were up-regulated in ovarian cancer and down-regulated in ovarian endometriosis. Furthermore, E2F4 and miR-1260b were down-regulated in ovarian endometriosis and up-regulated in PCOS. The miRNAs: miR-15b-5p, miR-26a-5p, miR-30c-5p were down-regulated in ovarian cancer and up-regulated in PCOS, while miR-423-3p and miR-93-3p were up-regulated in ovarian cancer and down-regulated in PCOS.

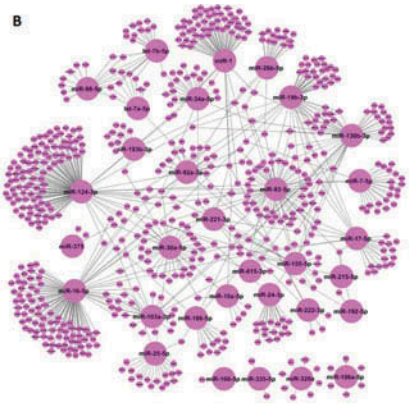
The common reporter TF-target DEG and miRNA-target DEG interactions were visualized (Figure 5A, B). Moreover, the target DEGs of reporter TFs and miRNAs were subjected to pathway enrichment analyses (Figure 5C). Accordingly, several cancer pathways (small cell lung, prostate, bladder, colorectal, and pancreatic cancers), p53 signaling pathway, progesterone-mediated oocyte maturation, and oocyte meiosis came into prominence when targets of ovarian cancer specific reporter TFs and miRNAs were investigated. Furthermore, Fc gamma R-mediated phagocytosis cancer pathways like bladder, prostate, and endometrial cancer as well as p53 signaling pathway were highlighted in ovarian endometriosis. Disease pathways for neurodegenerative diseases (i.e., Alzheimer's, Huntington's, Parkinson's diseases) were significantly enriched in PCOS (Figure 5D).

Discussion

Considering the crucial role of the ovary in the female reproductive system, the identification of functional biological entities (i.e., transcripts, miRNAs, proteins, metabolites, pathways) taking key roles in the mechanisms of ovarian-related diseases is a very challenging task. Though the relationships between endometriosis, ovarian cancer, and PCOS have been studied [Chittenden et al. 2009; Sayasneh et al. 2011; Pavone and Lyttle 2015], the molecular mechanisms behind their connections are not yet certain.

Although individual transcriptome studies have been performed for ovarian cancer, ovarian endometriosis, and PCOS, the gene expression datasets were not analyzed from an integrated and comparative perspective. Therefore, in the present study, we integrated disease specific gene expression datasets with comprehensive human biological networks in order to uncover central biological mechanisms behind the diseases.





C

Signaling and Regulatory Pathways	Reporter TFs	Reporter miRNAs
Adherens junction	+	+
Focal adhesion	+	+
Apoptosis	+	-
Progesterone-mediated oocyte maturation	+	-
Fc gamma R-mediated phagocytosis	+	-
Bladder, colorectal, endometrial, pancreatic and prostate cancers	+	+
Acute and chronic myeloid leukemia	+	+
Pathways in cancer	+	+
ErbB, mTOR and p53 signaling pathways	+	+
MAPK and TGF-beta signaling pathways	-	+

D

Disease	Signaling and Regulatory Pathways
Ovarian Cancer	Small cell lung, prostate, bladder, pancreatic, and colorectal cancers, p53 signaling pathway, progesterone-mediated oocyte maturation, oocyte meiosis
Ovarian Endometriosis	Prostate, bladder and endometrial cancers, p53 signaling pathway, Fc gamma R-mediated phagocytosis
PCOS	Alzheimer's, Huntington's and Parkinson's diseases

Figure 5. Transcriptional regulatory networks. (A) The network represents the interactions between the mutual reporter transcription factors (TFs) and their targeted differentially expressed genes (DEGs). (B) The network represents the interactions between the mutual reporter micro-RNAs (miRNAs) and their targeted DEGs. (C) The statistically significant ($p<0.05$) pathways enriched with DEGs targeted by mutual TFs and miRNAs. The presence and absence of statistical significance were represented by + and – symbols, respectively. (D) The statistically significant ($p<0.05$) pathways for DEGs targeted by reporter miRNAs and TFs for each disease.

By individual analyses of each gene expression dataset, hundreds of genes with statistically significant changes in their expression profiles were identified and further analyzed. Results of the statistical analyses demonstrated that the majority of the DEGs were down-regulated in ovarian cancer and PCOS. However, the number of down and up- regulated DEGs were close to each other in endometriosis. The comparative analyses of the DEGs indicated that 31 down- and six up-regulated genes were common among all three diseases. The central biological mechanisms of these ovarian related diseases were frequently down-regulated.

Reconstruction and analysis of biological networks (protein-protein interaction, transcriptional regulatory, and metabolic) represents a great potential to understand the central mechanisms of these diseases. Therefore, in the present study, we integrated results of statistical analyses with these biological networks to identify functional biological entities (i.e., transcripts, miRNAs, proteins, metabolites, pathways) taking key roles in the mechanisms of ovarian-related diseases. Initially, we reconstructed a PPI network and employed two topological parameters simultaneously to define central proteins (i.e., hubs), which can be considered as candidate biomarkers or drug targets. It was notable that hubs of investigated diseases have the potential to contribute formation or initiation of cancer development. Several hub proteins (i.e., PCNA, FYN, and SRC) have been associated with cancer development or progression [Moldovan et al. 2007; Saito et al. 2010; Sen and Johnson 2011]. In addition, these hub proteins had tumor suppressor (VHL and EGR1) [Lu et al. 2014; Kim et al. 2014], oncogenic (SIRT7 and IKBKE) [McGlynn et al. 2015; Hutti et al. 2009], and proto-oncogenic (MYC) functions in human cancers [Gabay et al. 2014]. Further, they also have roles in cancer linked signaling pathways such as TGF-β signaling pathway (SMAD2 and SMAD3) [Xue et al. 2014] and WNT/β-catenin signaling pathway (CTNNB1) [Bodnar et al. 2014]. The over-expression of AR protein in ovarian cancer, down-regulation of CTNNB1 and BRCA1 in ovarian endometriosis, and down-regulation of MAPK1 in PCOS were in line with previously reported studies [Govatati et al. 2014; Ortega and Duleba 2013].

Metabolism is an indicator of the proper functioning, thereby health, and metabolites are the intermediates and products of the metabolic reactions that inherently occur inside the cells. Thus, in the present study, we identified the reporter metabolites around which significant changes in gene expression patterns occur in disease conditions. Proliferation of cancer cells requires an extreme amount of energy whereby energy metabolism (i.e., the metabolism of glucose and other carbohydrates) plays a key role. It has been established that generally cancer cells generate their energy using glycolysis instead of oxidative phosphorylation even though oxygen is available for rapid growth [Vazquez et al. 2010]. Up-regulation of the NAD precursor and down-regulation of NAD leads to the proposal that cancer cells continuously produce NAD precursor and use NAD for energy production. Though endometriosis is a benign disease, but has a potential for malignant transformation [Pavone and Lyttle 2015], we encountered metabolites that were associated with cancer processes (apoptosis, cell survival) in ovarian endometriosis. In addition, identification of metabolites that were previously associated with prostate cancer (i.e., selenomethionine and choline) was noteworthy. Both endometriosis and prostate cancer can be considered sex-hormone related diseases, and even their treatment methods intersect at a certain point. For example, gonadotropin releasing hormone (GnRH) analogues such as leuprorelin and goserelin are both used in endometriosis and prostate cancer treatments [Wilson et al. 2007]. In the present study, we determined an association between ovarian endometriosis and prostate cancer at the metabolic level. Furthermore, reporter metabolite analyses indicated that metabolites that were related to oxidative stress/damage (i.e., ferricytocrome c and ubiquinol) were aberrant in PCOS. Oxidative stress is defined as a balance disturbance between the production of reactive oxygen species and antioxidant defenses. Obesity and insulin resistance can lead to an increase in the oxidative stress, and they are widespread in PCOS women [Desai et al. 2014]. Therefore, to encounter ferricytocrome c and ubiquinol in PCOS is a plausible consequence. Moreover, alanine and pyruvate were also dysregulated in PCOS. Overall these results reflected the alteration in energy production processes under PCOS.

Pathway information is essential for successful biological systems modelling. According to the results of pathway enrichment analyses, cell communication and connection pathways (i.e., adherens junction, ECM-receptor interaction, focal and cell adhesion) came into prominence. In ovarian cancer, adherens junction and focal adhesion pathways were under-expressed which might be due to the observation that tumor cells lose their adhesion properties. The focal adhesion pathway was up-regulated in ovarian endometriosis. It has been reported that focal adhesion associated protein (FAK) was over-expressed in ovarian endometriosis, and its possible contribution to disease pathogenesis and progression was proposed [Mu et al. 2008].

Signalling pathways and receptor interactions were altered in diseased cells, thus, as expected, the ECM-receptor interaction was over-expressed. Phagocytosis has a crucial role in host-defence mechanisms. Our results were in agreement with the proposal that a group of body protection systems should be activated in disease conditions [Neuberg et al. 2011]. Endometriosis has also been considered as an autoimmune disease, and increased cancer risk in autoimmune diseases such as systemic lupus was reported [Bernatsky et al. 2013]. Therefore, the significant enrichment among endometriosis, systemic lupus erythematous, and ovarian cancer is not surprising. Additionally, alterations in two signalling pathways (MAPK and Wnt) were highlighted. The MAPK signalling pathway has a crucial role in signal transduction and involved in comprehensive processes, including gene expression, hormone response, embryogenesis, and cell survival [Manna and Stocco 2011]. WNT signalling pathway plays a role in a variety of biological processes such as normal follicular development and ovarian functions, and it was reported that deviant WNT signalling underlies multifarious diseases [Clevers and Nusse 2012; Gatcliffe et al. 2008]. As expected, signal transduction pathways were indicating differences due to disease response. Already, the signalling pathways (MAPK and Wnt) were down-regulated in PCOS and this outcome is in accord with previous studies [Liu et al. 2015; Kenigsberg et al. 2009]. Sphingolipid metabolism, fatty acid metabolism, non-small cell lung cancer, prostate cancer, calcium signalling pathway, and phosphatidylinositol signalling system pathways were consistently under-expressed in ovarian cancer and endometriosis. In comparison, the regulatory directions of other pathways varied among the diseases. Disrupted sphingolipid metabolism was reported in several pathologies including proliferative, metabolic, and neuronal diseases [Rao et al. 2013]. Steroid hormones are synthesized and secreted from the ovary. Accordingly, in ovarian tissue related diseases we predict alterations in steroid hormone biosynthesis and fundamental building blocks like nucleic acids and amino acids. Alterations in associated pathways were not surprising. Aberrant calcium regulation has been associated with multiple diseases such as diabetes, hypertension, cardiovascular, Alzheimer, and cancer. Although changes in calcium signalling are not required for tumor inception, it was reported that impaired calcium signalling in tumor cells may support cancer metastasis [Chen et al. 2013].

Gene expression regulation in cells may be controlled by TFs and non-coding RNAs (such as miRNAs) either at the transcriptional and/or post-transcriptional levels, thus any alterations in these regulatory compounds may affect gene expression. Reporter TFs and miRNAs, and their target DEGs, have been identified in the present study, in order to discover the dynamics of the active transcriptional regulatory network in inspected diseases. It was found that the target genes of reporter TFs and miRNAs were involved in miscellaneous mutual signaling and regulatory pathways we encountered in this study (i.e., adherens junction, focal adhesion, Fc gamma R mediated phagocytosis, and MAPK signaling pathway). Apart from these pathways, certain major signaling pathways were distinguished including ErbB, mTOR, p53, and TGF-β. It was reported that members of the ErbB family are present in a few types of cancer, up-regulation of ErbBs is associated with decreasing survival, and ErbB directed therapies are frequently targeted for cancer treatment [Schmukler and Pinkas-Kramarski 2015]. The mTOR signaling pathway has been frequently dysregulated in human cancers including ovarian, breast, colon, renal, and head and neck, thus there is a considerable concern in examining mTOR as a therapeutic target for cancers [Pópulo et al. 2012]. TGF-β can act as both a tumor suppressor and a promoter depending on context. The TGF-β signaling pathway has been suggested as a target for cancer therapy having shown encouraging outcomes in varied cancers [Nagaraj and Datta 2010]. Moreover, either internal or external stress signals (which may cause DNA damage) may activate the p53 pathway. According to stress signals, p53 protein is activated by post-translational modifications, and this lead to cell cycle arrest, cellular senescence, or apoptosis [Harris and Levine 2005]. Consequently, it is feasible to encounter these signal transduction pathways with ovarian cancer as therapeutic targets.

Ovarian cancer and ovary related diseases represent complex pathology outcomes from genetic alterations and environmental influences. Understanding the disease pathways requires omics data from several levels (especially, proteomics and metabolomics). However, experimental data to analyze the biological mechanisms of ovarian diseases are very limited, even at the transcriptomic level. The systems network biomedicine approach used here employs genome-scale biological networks, which are reconstructed via high-throughput datasets. Further detail awaits the analysis of the data afforded by Next Generation approaches including sequencing. This should improve the quality of data employed in the network reconstruction and thus increase the prediction accuracy of the network-based analyses. In the present analysis, sample sizes of transcriptomic datasets were limited. Although power analyses indicated the sufficiency of sample sizes for the

employed datasets, increments in the sample sizes will also improve the efficiency of the statistical methods, especially in determination of differentially expressed genes.

In conclusion, comparative and integrative analysis of transcriptome datasets for ovarian cancer, ovarian endometriosis, and PCOS resulted with several proteomic, metabolic, and transcriptional regulatory signatures for further analyses. These signatures, overall, were associated with formation or initiation of cancer development, and pointed out the potential tendency of PCOS and endometriosis to tumorigenesis. Molecules and pathways related to MAPK signaling, cell cycle, and apoptosis were the mutual determinants in pathogenesis of all three diseases. This study provides signatures which could be considered as candidates for the design of diagnostic tools and treatment strategies for further experimental and clinical studies. This study contributed to the molecular signatures which may be used for screening or therapeutic purposes, and may be beneficial for understanding etiopathogenesis and biological mechanisms of ovarian diseases.

Materials and methods

A multi-stage analysis method was developed and applied here (Figure 1). Initially, gene expression datasets were statistically analyzed and differentially expressed genes and their regulatory patterns were identified. Considering DEG sets, functional enrichment studies were performed to identify enriched pathways, annotation terms (i.e., Gene Ontology terms), diseases, and biological processes. Then, results were integrated with biological networks to identify reporter biomolecules.

High-throughput gene expression datasets

To analyze the gene expression profiles of ovarian tissue diseases (ovarian cancer, endometriosis, and PCOS), six independently high throughput datasets (GSE7463, GSE14407, GSE7305, GSE1615a, GSE1615b, and GSE10946- lean samples) were downloaded from the Gene Expression Omnibus (GEO) database [Barrett et al. 2013]. A total of 92 (46 diseased, 46 control) samples were examined, including 21 samples from ovarian cancer and 22 non-ovarian cancer tissues, 10 diseased and 10 healthy samples for ovarian endometriosis, and 15 diseased and 14 healthy samples for PCOS (Table 1).

Identification of differentially expressed genes

Each dataset was statistically analyzed to identify DEGs. The raw.CEL microarray files were normalized through computing the Robust Multi-Array Average (RMA) expression measure [Bolstad et al. 2003] as implemented in the affy package [Gautier et al. 2004] of R/Bioconductor platform (version Rx64 3.0.2) [Gentleman et al. 2004]. DEGs were identified from the normalized expression values using Linear Models for Microarray Data (LIMMA) package [Smyth 2005]. To determine statistical significance of the DEGs, p-value threshold was used ($p<0.01$), and fold changes were taken into account to determine the regulatory patterns of the DEGs. The required ID conversions were obtained from Biodbnet platform [Mudunuri et al. 2009]. Since transcriptome data employed in the present study were obtained from various ovarian cells and tissues, each dataset was statistically analyzed independently; then, a unique DEG list was obtained for each disease with multiple datasets (i.e., ovarian cancer and PCOS) by taking the union of DEG sets derived from each dataset.

Reconstruction of protein-protein interaction networks and topological analysis

The previously reconstructed PPI network of *Homo sapiens*, which consists of 288,033 physical interactions between 21,052 proteins, was recruited in the present study [Karagoz et al. 2015]. For each dataset, a PPI subnetwork was reconstructed around DEGs, represented as undirected graphs (i.e., nodes represent proteins and the edges represent interactions between the proteins), visualized and analyzed via Cytoscape (v2.8.3) [Smoot et al. 2011]. To determine hub proteins, topological analyses were applied through Cyto-Hubba plugin [Smoot et al. 2011] and the dual-metric approach [Karagoz et al. 2015; Calimlioglu et al. 2015] considering degree and betweenness centrality measures simultaneously was employed. The top 5% of the proteins with the highest degree and/or betweenness centrality in the network were presented as hub proteins.

Determination of disease specific reporter metabolites

The genome-scale metabolic network, which provides information about metabolic reactions and the genes that encode enzymes catalyzing these reactions, was gained from the latest version of the Human Metabolic Reaction (HMR 2.00) model which is available from Human Metabolic Atlas resource [Mardinoglu et al. 2014]. The reporter metabolites (around which significant gene expression changes occur) were identified via BioMet Toolbox (v2.0) by

using up and down-regulated DEGs of each disease [Garcia-Albornoz et al. 2014]. *P*-value threshold ($p<0.05$) was used to determine statistical significance.

Enrichment analyses of DEGs and reporter metabolites

Disease, biological process, molecular function, and pathway enrichment analyses of DEGs were performed via DAVID's functional annotation tool [Huang et al. 2009]. In the analyses, Genetic Association Database [Becker et al. 2004] and KEGG [Kanehisa et al. 2014] were preferably employed as disease and pathway databases, respectively. Functional enrichment analysis for metabolites was performed via MBRole [Chagoyen and Pazos 2011]. Fisher's exact test was used to evaluate the significance of the enrichments and a *p*-value threshold of $p<0.05$ was used for all enrichment analyses.

Determination of reporter transcription factors and miRNAs

Reporter TFs and miRNAs were determined by using experimentally verified TF-target gene interactions downloaded from PAZAR database [Portales-Casamar et al. 2009], and experimentally supported miRNA-target gene interactions downloaded from miRTarbase (Release 4.5) [Hsu et al. 2014]. The reporter features algorithm [Patil and Nielsen 2005] was employed and implemented in MATLAB (R2010) [Sevimoglu and Arga 2015]. The z-scores following a standard normal distribution was converted to *p*-values, and statistically significant ($p<0.05$) TFs and miRNAs were considered to be the reporter transcriptional regulatory components. Specific and common regulatory components in each disease were identified and target DEGs of common reporter TFs and miRNAs were extracted from the TF-miRNA-gene interaction network constructed in our previous study [Calimlioglu et al. 2015]. The target DEGs of reporter TFs and miRNAs were subjected to pathway enrichment analyses through DAVID's functional annotation tool [Huang et al. 2009].

Acknowledgments

The authors thank the Editor, Reviewers, and Ahmet Kaplan for their significant contributions during the revision period.

Declaration of interest

Financial support by Marmara University, Scientific Research Projects Committee (BAPKO) through project FEN-C-DRP-110915-0445. The authors declare no conflicts of interest.

Notes on contributors

Analyzed the data and evaluated the results: MK; Designed the algorithms and the analysis framework: EG, KYA; Conceived and directed the study: KYA; Wrote the paper: MK, EG, KYA. All authors read and approved the final version.

References

Anam, M.T., Ishika, A., Hossain, M.B. and Jesmin (2015) A meta-analysis of hypoxia inducible factor 1-alpha (HIF1A) gene polymorphisms: association with cancers. Biomark Res **3**: 29.

Aragon-Ching, J.B. (2014) The evolution of prostate cancer therapy: targeting the androgen receptor. Front Oncol **4**: 295.

Atiomo, W. and Daykin, C.A. (2012) Metabolomic biomarkers in women with polycystic ovary syndrome: a pilot study. Mol Hum Reprod **18**(11): 546–553.

Aviel-Ronen, S., Soriano, D., Shmuel, E., Schonman, R., Rosenblatt, K., Zadok, O., et al. (2014) Surgically treated ovarian endometriosis association with BRCA1 and BRCA2 mutations. Pathol Res Pract **210**(4): 250–255.

Awwad, H.M., Geisel, J. and Obeid, R. (2012) The role of choline in prostate cancer. Clin Biochem **45**(18): 1548–1553.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H.F., Futterweit, W., et al. (2009) The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril **91**(2): 456–488.

Bain, M.A., Faull, R., Fornasini, G., Milne, R.W. and Evans, A.M. (2006) Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. Nephrol Dial Transplant **21**(5): 1300–1304.

Barabási, A.L., Gulbahce, N. and Loscalzo, J. (2011) Network medicine: a network-based approach to human disease. Nat Rev Genet **12**(1): 56–68.

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., et al. (2013) NCBI GEO: archive for functional genomics data sets-update. Nucleic Acids Res **41**: 991–995.

Becker, K.G., Barnes, K.C., Bright, T.J. and Wang, S.A. (2004) The genetic association database. Nat Genet **36**(5): 431–432.

Beckerman, R. and Prives, C. (2010) Transcriptional regulation by p53. Cold Spring Harb Perspect Biol **2**(8): a000935.

Bellelis, P., Podgaec, S. and Abrao, M.S. (2011) Environmental factors and endometriosis. Rev Assoc Med Bras **57**(4): 448–452.

Bento, C., Andersson, M.K. and Aman, P. (2009) DDIT3/CHOP and the sarcoma fusion oncoprotein FUS-DDIT3/TLS-CHOP bind cyclin-dependent kinase 2. BMC Cell Biol **10**:89.

Bernatsky, S., Ramsey-Goldman, R., Labrecque, J., Joseph, L., Boivin, J.F., Petri, M., et al. (2013) Cancer risk in systemic lupus: an updated international multi-centre cohort study. J Autoimmun **42**:130-135.

Bodnar, L., Stanczak, A., Cierniak, S., Smoter, M., Cichowicz, M., Kozlowski, W., et al. (2014) Wnt/β-catenin pathway as a potential prognostic and predictive marker in patients with advanced ovarian cancer. J Ovarian Res **7**:16.

Bolstad, B.M., Irizarry, R.A., Astrand, M. and Speed, T.P. (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics **19**(2): 185–193.

Bowen, N.J., Walker, L.D., Matyunina, L.V., Logani, S., Totten, K.A., Benigno, B.B., et al. (2009) Gene expression profiling supports the hypothesis that human ovarian surface epithelia are multipotent and capable of serving as ovarian cancer initiating cells. BMC Med Genomics **29**(2):71.

Calimlioglu, B., Karagoz, K., Sevimoglu, T., Kilic, E., Gov, E. and Arga, K.Y. (2015) Tissue-specific molecular biomarker signatures of Type 2 Diabetes: An integrative analysis of transcriptomics and protein–protein interaction data. OMICS **19**(9): 563–573.

Cathcart, M.C., Gray, S.G., Baird, A.M., Boyle, E., Gately, K., Kay, E., et al. (2011) Prostacyclin synthase expression and epigenetic regulation in nonsmall cell lung cancer. Cancer **117**(22): 5121–5132.

Chagoyen, M. and Pazos, F. (2011) MBRole: enrichment analysis of metabolomic data. Bioinformatics **27**(5):730–731.

Chang, C., Lee, S.O., Wang, R.S., Yeh, S. and Chang, T.M. (2013a). Androgen receptor (AR) physiological roles in male and female reproductive systems: lessons learned from AR-knockout mice lacking AR in selective cells. Biol Reprod **89**(1): 1–16.

Chang, J.H., Au, H.K., Lee, W.C., Chi, C.C., Ling, T.Y., Wang, L.M., et al. (2013b) Expression of the pluripotent transcription factor OCT4 promotes cell migration in endometriosis. Fertil Steril **99**(5):1332–1339.

Chen, L., Wang, S., Zhou, Y., Wu, X., Entin, I., Epstein, J., et al. (2010) Identification of early growth response protein 1 (EGR-1) as a novel target for JUN-induced apoptosis in multiple myeloma. Blood **115**(1): 61–70.

Chen, Y.F., Chen, Y.T., Chiu, W.T. and Shen, M.R. (2013) Remodeling of calcium signaling in tumor progression. J Biomed Sci **20**: 23.

Chi, Y. and Sauve, A.A. (2013) Nicotinamide riboside, a trace nutrient in foods, is a vitamin B3 with effects on energy metabolism and neuroprotection. Curr Opin Clin Nutr Metab Care **16**(6): 657–661.

Chiarugi, A., Dölle, C., Felici, R. and Ziegler, M. (2012) The NAD metabolome–a key determinant of cancer cell biology. Nat Rev Cancer **12**(11): 741–752.

Chittenden, B.G., Fullerton, G., Maheshwari, A. and Bhattacharya, S. (2009) Polycystic ovary syndrome and the risk of gynecological cancer: a systematic review. Reprod Biomed Online **19**(3): 398–405.

Clevers, H. and Nusse, R. (2012) Wnt/β-catenin signaling and disease. Cell **149**(6): 1192–1205.

Desai, V., Prasad, N.R., Manohar, S.M., Sachan, A., Narasimha, S.R. and Bitla, A.R. (2014) Oxidative stress in non-obese women with polycystic ovarian syndrome. J Clin Diagn Res **8**(7): 1–3.

Didžiapetrienė, J., Bublevič, J., Smailytė, G., Kazbarienė, B. and Stukas, R. (2014) Significance of blood serum catalase activity and malondialdehyde level for survival prognosis of ovarian cancer patients. Medicana **50**: 204–208.

Dittmer, J. (2003) The biology of the Ets1 proto-oncogene. Mol Cancer **2**:29.

Filigheddu, N., Gregnanin, I., Porporato, P.E., Surico, D., Perego, B., Galli, L., et al. (2010) Differential expression of microRNAs between eutopic and ectopic endometrium in ovarian endometriosis. J Biomed Biotechnol 2010: 369549.

Fong, M.Y., McDunn, J. and Kakar, S.S. (2011) Identification of metabolites in the normal ovary and their transformation in primary and metastatic ovarian cancer. PLoS One **6** (5): e19963.

Gabay, M., Li, Y. and Felsher, D.W. (2014) MYC activation is a hallmark of cancer initiation and maintenance. Cold Spring Harb Perspect Med **4**(6): a014241.

Garcia-Albornoz, M., Thankaswamy-Kosalai, S., Nilsson, A., Varemo, L., Nookaew, I. and Nielsen, J. (2014) BioMet Toolbox 2.0: genome-wide analysis of metabolism and omics data. Nucleic Acids Res **42**:175-181.

Gatcliffe, T.A., Monk, B.J., Planutis, K. and Holcombe, R.F. (2008) Wnt signaling in ovarian tumorigenesis. Int J Gynecol Cancer **18**(5): 954–962.

Gatt, S. and Dagan, A. (2012) Cancer and sphingolipid storage disease therapy using novel synthetic analogs of sphingolipids. Chem Phys Lipids **165**(4): 462–474.

Gautier, L., Cope, L., Bolstad, B.M. and Irizarry, R.A. (2004) Affy- analysis of Affymetrix GeneChip data at the probe level. Bioinformatics **20**(3): 307–315.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol **5**(10): 80.

Gibellini, F. and Smith, T.K. (2010) The Kennedy pathway–De novo synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB Life **62**(6): 414–428.

Govatati, S., Challa, K., Reddy, S.B., Pramod, K., Deenadayal, M., Chakravarty, B., et al. (2014) BRCA1 alterations are associated with endometriosis, but BRCA2 alterations show no detectable endometriosis risk: a study in Indian population. J. Assist. Reprod Genet **32**(2): 277–285.

Goyama, S., Schibler, J., Cunningham, L., Zhang, Y., Rao, Y. and Nishimoto, N. (2013) Transcription factor RUNX1 promotes survival of acute myeloid leukemia cells. J Clin Invest **123**(9): 3876–3888.

Harris, H.R., Cramer, D.W., Vitonis, A.F., DePari, M. and Terry, K.L. (2012) Folate, vitamin B6, vitamin B12, methionine and alcohol intake in relation to ovarian cancer risk. Int J Cancer **131**(4): 518–529.

Harris, S.L. and Levine, A.J. (2005) The p53 pathway: positive and negative feedback loops. Oncogene **24**(17): 2899–2908.

Hatakeyama, S. (2011) TRIM proteins and cancer. Nature Reviews Cancer **11**: 792–804.

Hayes, J., Peruzzi, P.P., and Lawler, S. (2014) MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med **20**(8): 460–469.

Hever, A., Roth, R.B., Hevezi, P., Marin, M.E., Acosta, J.A., Acosta, H., et al. (2007) Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. Proc Natl Acad Sci U S A **104**(30): 12451–12456.

Hira, S., Tomita, T., Matsui, T., Igarashi, K. and Ikeda-Saito, M. (2007) Bach1, a heme-dependent transcription factor, reveals presence of multiple heme binding sites with distinct coordination structure. IUBMB Life **59**(8–9): 542–551.

Hsu, S.D., Tseng, Y.T., Shrestha, S., Lin, Y.L., Khaleel, A., Chou, C.H., et al. (2014) miRTarBase update 2014: an information resource for experimentally validated miRNA-target interactions. Nucleic Acids **42**: 78–85.

Huang, D.W., Sherman, B.T. and Lempicki, R.A. (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res **37**(1): 1–13.

Hutti, J.E., Shen, R.R., Abbott, D.W., Zhou, A.Y., Sprott, K.M., Asara, J.M., et al. (2009) Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKK-epsilon promotes cell transformation. Mol Cell **34**: 461–472.

Iorio, M.V., Visone, R., Di Leva, G., Donati, V., Petrocca, F., Casalini, P., et al. (2007) MicroRNA signatures in human ovarian cancer. Cancer Res **67**(18): 8699–8707.

Iwanaga, R., Komori, H., Ishida, S., Okamura, N., Nakayama, K., Nakayama, K.I., et al. (2006) Identification of novel E2F1 target genes regulated in cell cycle-dependent and independent manners. Oncogene **25**(12): 1786–798.

Jiang, L., Huang, J., Li, L., Chen, Y., Chen, X., Zhao, X., et al. (2015) MicroRNA-93 promotes ovarian granulosa cells proliferation through targeting CDKN1A in polycystic ovarian syndrome. J Clin Endocrinol Metab **100**(5): 729–738.

Johansson, J. and Stener-Victorin, E. (2013) Polycystic ovary syndrome: effect and mechanisms of acupuncture for ovulation induction. Evid Based Complement Alternat Med 2013: 762615.

Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumich, M. and Tanabe, M. (2014) Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res **42**: 199–205.

Karagoz, K., Sinha, R. and Arga, K.Y. (2015) Triple negative breast cancer: a multi-omics network discovery strategy for candidate targets and driving pathways. OMICS **19**(2): 115–130.

Ke, C., Hou, Y., Zhang, H., Fan, L., Ge, T., Guo, B., et al. (2015) Large-scale profiling of metabolic dysregulation in ovarian cancer. Int J Cancer **136**(3): 516–526.

Kenigsberg, S., Bentov, Y., Chalifa-Caspi, V., Potashnik, G., Ofir, R. and Birk, O.S. (2009) Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. Mol Hum Reprod **15** (2): 89–103.

Kim, J., Kang, H.S., Lee, Y.J., Lee, H.J., Yun, J., Shin, J.H., et al. (2014) EGR1-dependent PTEN upregulation by 2-benzoyloxycinnamaldehyde attenuates cell invasion and EMT in colon cancer. Cancer Lett **349**(1): 35–44.

Lamarre, S.G., Morrow, G., Macmillan, L., Brosnan, M.E. and Brosnan, J.T. (2013) Formate: an essential metabolite, a biomarker, or more? Clin Chem Lab Med **51**(3): 571–578.

Laudanski, P., Charkiewicz, R., Kuzmicki, M., Szamatowicz, J., Charkiewicz, A. and Niklinski, J. (2013) MicroRNAs expression profiling of eutopic proliferative endometrium in women with ovarian endometriosis. Reprod Biol Endocrinol **11**: 78.

Li, H., Xie, S., Liu, M., Chen, Z., Liu, X., Wang, L., et al. (2014) The clinical significance of downregulation of mir-124-3p, mir-146a-5p, mir-155-5p and mir-335-5p in gastric cancer tumorigenesis. Int J Oncol **45**(1): 197–208.

Ligr, M., Patwa, R.R., Daniels, G., Pan, L., Wu, X., Li, Y., et al. (2011) Expression and function of androgen receptor coactivator p44/Mep50/WDR77 in ovarian cancer. PLoS One **6**(10): e26250

Liu, S., Zhang, X., Shi, C., Lin, J., Chen, G., Wu, B., et al. (2015) Altered microRNAs expression profiling in cumulus cells from patients with polycystic ovary syndrome. J Transl Med **13**: 238.

Llauradó, M., Abal, M., Castellví, J., Cabrera, S., Gil-Moreno, A., Pérez-Benavente, A., et al. (2012) ETV5 transcription factor is over-expressed in ovarian cancer and regulates cell adhesion in ovarian cancer cells. Int J Cancer **130**(7): 1532–1543.

Lu, Y.Y, Zhu, W.J. and Xie, B.G. (2014) Von Hippel-Lindau gene expression in human endometrium during menstrual cycle. Mol Med Rep **9**(4): 1355–1358.

Manna, P.R. and Stocco, D.M. (2011) The Role of Specific Mitogen-Activated Protein Kinase Signaling Cascades in the Regulation of Steroidogenesis. J Signal Transduct 2011: 821615.

Mardinoglu, A., Agren, R., Kampf, C., Asplund, A., Uhlen, M. and Nielsen, J. (2014) Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease. Nat Commun **5**: 3083.

McGinnis, L.K., Kinsey, W.H. and Albertini, D.F. (2009) Functions of Fyn kinase in the completion of meiosis in mouse oocytes. Dev Biol **327**(2): 280–287.

McGlynn, L.M., McCluney, S., Jamieson, N.B., Thomson, J., MacDonald, A.I., Oien, K., et al. (2015) SIRT3 & SIRT7: Potential novel biomarkers for determining outcome in pancreatic cancer patients. PLoS One **10**(6): e0131344.

Meresman, G.F. and Olivares, C.N. (2012) Involvement of Prostaglandins in the Pathophysiology of Endometriosis. In Endometriosis - Basic Concepts and Current Research Trends ed. by Chaudhury, K and Chakravarty, B., InTech, Croatia. pp. 115–132.

Miles, L., Miles, M.V., Tang, P.H., Horn, P.S., Quinlan, J.G., Wong, B., et al. (2005) Ubiquinol: a potential biomarker for tissue energy requirements and oxidative stress. Clin Chim Acta **360**(1–2): 87–96.

Mills, E. and O'Neill, L.A. (2014) Succinate: a metabolic signal in inflammation. Trends Cell Biol **24**(5): 313–320.

Mitchell, T.J. and John, S. (2005) Signal transducer and activator of transcription (STAT) signalling and T-cell lymphomas. Immunology **114**(3): 301–312.

Moldovan, G.L., Pfander, B. and Jentsch, S. (2007) PCNA, the maestro of the replication fork. Cell **129**: 665–679.

Moreno, C.S., Matyunina, L., Dickerson, E.B., Schubert, N., Bowen, N.J., Logani, S., et al. (2007) Evidence that p53-mediated cell-cycle-arrest inhibits chemotherapeutic treatment of ovarian carcinomas. PLoS One **2**(5): e441.

Mu, L., Zheng, W., Wang, L., Chen, X. and Yang J. (2008) Focal adhesion kinase expression in ovarian endometriosis. Int J Gynaecol Obstet **101**(2): 161–165.

Mudunuri, U., Che, A., Yi, M. and Stephens, R.M. (2009) bioDBnet: the biological database network. Bioinformatics **25**: 555–556.

Muñoz-Pinedo, C., El Mjiyad, N. and Ricci, J.E. (2012) Cancer metabolism: current perspectives and future directions. Cell Death Dis **3**(1): e248.

Nagaraj, N.S. and Datta, P.K. (2010) Targeting the transforming growth factor-beta signaling pathway in human cancer. Expert Opin Investig Drugs **19**(1): 77–91.

Nahum, G.G., Kaunitz, A.M., Rosen, K., Schmelter, T. and Lynen, R. (2015) Ovarian cysts: presence and persistence with use of a 13.5mg levonorgestrel-releasing intrauterine system. Contraception **91**(5): 412–417

Neuberg, S.L., Kenrick, D.T. and Schaller, M. (2011) Human threat management systems: self-protection and disease avoidance. Neurosci Biobehav Rev **35**(4): 1042–1051.

Nishikawa, N., Toyota, M., Suzuki, H., Honma, T., Fujikane, T., Ohmura, T., et al. (2007) Gene amplification and overexpression of PRDM14 in breast cancers. Cancer Res **67** (20): 9649–9657.

Nyman, D.W., Suzanne Stratton, M., Kopplin, M.J., Dalkin, B.L., Nagle, R.B. and Jay Gandolfi, A. (2004) Selenium and selenomethionine levels in prostate cancer patients. Cancer Detect Prev **28**(1): 8–16.

Oksuz, Z., Serin, M.S, Kaplan, E., Dogen, A., Tezcan, S., Aslan, G., et al. (2015) Serum microRNAs; miR-30c-5p, miR-223-3p, miR-302c-3p and miR-17-5p could be used as novel non-invasive biomarkers for HCV-positive cirrhosis and hepatocellular carcinoma. Mol Biol Rep **42**(3): 713–720.

Ortega, I. and Duleba, A.J. (2013) Polycystic Ovary Syndrome: Current and Emerging Concepts: Role of Statins. In PCOS Management ed. Pal, L. Springer Science, New York. pp. 182–203.

Patil, K.R. and Nielsen, J. (2005) Uncovering transcriptional regulation of metabolism by using metabolic network topology. Proc Natl Acad Sci U S A **102**(8): 2685–2689.

Pavone, M.E. and Lyttle, B.M. (2015) Endometriosis and ovarian cancer: links, risks, and challenges faced. Int J Womens Health **7**: 663–672.

Pópulo, H., Lopes, J.M. and Soares, P. (2012) The mTOR signalling pathway in human cancer. Int J Mol Sci **13**(2): 1886–1918.

Portales-Casamar, E., Arenillas, D., Lim, J., Swanson, M.I., Jiang, S., McCallum, A., et al. (2009) The PAZAR database of gene regulatory information coupled to the ORCA toolkit for the study of regulatory sequences. Nucleic Acids Res **37**: 54–60.

Prat, J. (2014) Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynecol Obstet **124**: 1–5.

Pundir, C.S. and Devi, R. (2014) Biosensing methods for xanthine determination: a review. Enzyme Microb Technol **10**(57): 55–62.

Rao, R.P., Vaidyanathan, N., Rengasamy, M., Oommen, A. M., Somaiya, N. and Jagannath, M.R. (2013) Sphingolipid Metabolic Pathway: An Overview of Major Roles Played in Human Diseases. J Lipids 2013: 178910.

Richards, J.S. and Pangas, S.A. (2010) The ovary: basic biology and clinical implications. J Clin Invest **120**(4): 963–972.

Saito, Y.D., Jensen, A.R., Salgia, R., Edwin M. and Posadas, E. M. (2010) Fyn: a novel molecular target in prostate cancer. Cancer **116**(7): 1629–1637.

Sampson, J. A. (1925) Endometrial carcinoma of the ovary, arising in endometrial tissue in that organ. Arch Surg. **10**(1).

Sayasneh, A., Tsivos, D. and Crawford, R. (2011) Endometriosis and ovarian cancer: a systematic review. ISRN Obstet Gynecol **2011**:140310.

Schmukler, E. and Ronit Pinkas-Kramarski R. (2015) Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging: Role in Human Diseases ed. Hayat, M.A., Elsevier Inc., MA. pp. 65–80.

Sedlák, E., Fabian, M., Robinson, N.C. and Musatov, A. (2010) Ferricytochrome c protects mitochondrial cytochrome c oxidase against hydrogen peroxide-induced oxidative damage. Free Radic Biol Med **49**(10): 1574–1581.

Sen, B. and Johnson, F.M. (2011) Regulation of SRC family kinases in human cancers. J Signal Transduct 2011: 865819.

Sevimoglu, T. and Arga, K.Y. (2015) Computational systems biology of psoriasis: Are we ready for the age of omics and systems biomarkers? OMICS **19**(11): 669–687.

Shi, Y. and Massague, J. (2003) Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell **113**: 685–700.

Signorile, P.G. and Baldi, A. (2015) New evidence in endometriosis. Int J Biochem Cell Biol **60**: 19–22.

Simoens, S., Hummelshoj, L. and D'Hooghe, T. (2007) Endometriosis: cost estimates and methodological perspective. Hum Reprod Update **13**(4): 395–404.

Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L. and Ideker, T. (2011) Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics **27**(3): 431–432.

Smyth, G.K. (2005) Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor. eds. Gentleman, R., Carey, V., Dudoit, S., Irizarry, R., Huber, W. (eds), Springer, NY pp. 397–420.

Sørensen, A.E., Wissing, M.L., Salö, S., Englund, A.L.M. and Dalgaard, L.T. (2014) MicroRNAs related to polycystic ovary syndrome (PCOS). Genes **5**: 684–708.

Stevens, V.L., McCullough, M.L., Pavluck, A.L., Talbot, J.T., Feigelson, H.S., Thun, M.J., et al. (2007) Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol Biomarkers Prev **16**(6): 1140–1147.

Stoimenov, I. and Helleday, T. (2009) PCNA on the crossroad of cancer. Biochem Soc Trans **37**: 605–613.

Su, H.L. and Li, S.S. (2002) Molecular features of human ubiquitin-like SUMO genes and their encoded proteins. Gene **296**(1–2): 65–73.

Sun, B. and Jiang, H. (2012) An efficient approach for total synthesis of aminopropyl functionalized ganglioside GM1b. Tetrahedron Lett **53**(42): 5711–5715.

Vazquez, A., Liu, J., Zhou, Y. and Oltvai, Z.N. (2010) Catabolic efficiency of aerobic glycolysis: The Warburg effect revisited. BMC Syst Biol **4**:58.

Vouk, K., Hevir, N., Ribić-Pucelj, M., Haarpaintner, G., Scherb, H., Osredkar, J., et al. (2012) Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis. Hum Reprod **27**(10): 2955–2965.

Wang, H.L., Lu, R.Q., Xie, S.H., Zheng, H., Wen, X.M., Gao, X., et al. (2015) SIRT7 exhibits oncogenic potential in human ovarian cancer cells. Asian Pac J Cancer Prev **16** (8): 3573–3577.

Whitworth, J.M., Londoño-Joshi, A.I., Sellers, J.C, Oliver, P.J., Muccio, D.D., Atigadda, V.R., et al. (2012) The impact of novel retinoids in combination with platinum chemotherapy on ovarian cancer stem cells. Gynecol Oncol **125**(1): 226–230.

Wilson, A.C., Meethal, S.V., Bowen, R.L. and Atwood, C.S. (2007) Leuprolide acetate: a drug of diverse clinical applications. Expert Opin Investig Drugs **16**(11): 1851–1863.

Wood, J.R., Nelson-Degrave, V.L., Jansen, E., McAllister, J. M., Mosselman, S. and Strauss, J.F. 3rd (2005) Valproate-induced alterations in human theca cell gene expression: clues to the association between valproate use and metabolic side effects. Physiol Genomics **20**(3): 233–243.

Xue, J., Lin, X., Chiu, W.T., Chen, Y.H., Yu, G., Liu, M., et al. (2014) Sustained activation of SMAD3/SMAD4 by FOXM1 promotes TGF-β–dependent cancer metastasis. J Clin Invest **124**(2): 564–579.

Yang, Y., Hou, N., Wang, X., Wang, L., Chang, S., He, K., et al. (2015) miR-15b-5p induces endoplasmic reticulum stress and apoptosis in human hepatocellular carcinoma, both in vitro and in vivo, by suppressing Rab1A. Oncotarget **6**(18): 16227–16238.

Yang, Z., He, M., Wang, K., Sun, G., Tang, L. and Xu, Z. (2014) Tumor suppressive microRNA-193b promotes breast cancer progression via targeting DNAJC13 and RAB22A. Int J Clin Exp Pathol **7**(11): 7563–7570.

Ye, X. and Chun, J. (2010) Lysophosphatidic acid (LPA) signaling in vertebrate reproduction. Trends Endocrinol Metab **21**(1): 17–24.

Yu, D., Kim, M., Xiao, G. and Hwang, T.H. (2013) Review of biological network data and its applications. Genomics Inform **11**(4): 200–210.

Zhang, J., Song, Y., Zhang, C., Zhi, X., Fu, H., Ma, Y., et al. (2015) Circulating MiR-16-5p and MiR-19b-3p as Two Novel Potential Biomarkers to Indicate Progression of Gastric Cancer. Theranostics **5**(7): 733–745.

Zhang, L., Liu, W., Hao, Q., Bao, L. and Wang, K. (2012) Folate intake and methylenetetrahydrofolate reductase gene polymorphisms as predictive and prognostic biomarkers for ovarian cancer risk. Int J Mol Sci **13**(4): 4009–4020.