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Transcriptomic Analysis of Human Ovarian Cancer Cells: Changes Mediated by Luteinizing Hormone Receptor Activation

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1. Introduction

According to the American Cancer Society (American Cancer Society, 2011), there were approximately 21,880 new cases of ovarian cancer in the US in 2010, representing ~3% of all cases of newly diagnosed cancer. It is estimated that 13,850 women died from the disease in the same year, thus ranking ovarian cancer as the fifth leading cause of cancer death in the US, only behind lung, breast, colorectal, and pancreatic. These five cancers account for over 60% of cancer deaths in the US. The high mortality rate associated with ovarian cancer is attributable largely to its diagnosis at later stages of progression (Choi et al., 2007) when treatment options are limited and often ineffectual. In the early stages of the disease, most patients are asymptomatic or exhibit rather non-specific symptoms or discomfort.

Epidemiological evidence has established that risk factors for ovarian cancer include a family history of ovarian or breast cancer, often arising from mutations in the BRCA1 or the BRCA2 gene, occurrence of breast cancer, again often due to the same mutations, high body weight, and the use of just estrogen without added progesterone for postmenopausal hormone therapy. Some protection appears to arise from oral contraceptives, pregnancy, tubal ligation, and perhaps hysterectomy (American Cancer Society, 2011).

The etiology of ovarian cancer is overall poorly understood. There is, however, a prevailing theory that the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), may be contributory to the development or progression of the disease. The gonadotropins are heterodimeric glycoprotein hormones characterized by a common α-subunit and a hormone specific β-subunit. Both LH and FSH have been shown to have numerous effects on cultured ovarian carcinoma cells (Choi et al., 2007; Leung & Choi, 2007; Mandai et al., 2007; Lau et al., 2010). Moreover, the G protein-coupled receptors for LH (LHR) and FSH (FSHR) are expressed in the epithelial cells of the ovary (Choi et al., 2007). In the postmenopausal years, serum concentrations of LH and FSH are high due to the lack of negative feedback to the hypothalamus and pituitary concomitant with cessation of ovarian

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function. The structural and functional aspects of gonadotropins and their receptors have been recently reviewed (Ascoli & Puett, 2009).

In contrast to the above suggestion that gonadotropins are involved in the initiation or progression of the disease, there are clinical reports showing very little evidence that the use of gonadotropins to treat infertility increases the risk of ovarian cancer (Mosgaard et al., 1997; Sanner et al., 2009). Considering the available data, including gonadotropin ablation with gonadotropin-releasing hormone (GnRH) analogs, the conclusion was reached that if gonadotropins are involved in ovarian cancer, their role is probably more important in tumorigenesis and early growth, not in later stages (Huhtaniemi, 2010).

In this controversial area surrounding gonadotropins and ovarian cancer, there are a number of mixed, often conflicting, reports on established ovarian cancer cell lines regarding the actions of gonadotropins on cell proliferation, invasion, and migration (Choi et al., 2007). Consequently, a thorough examination of LH action on gene expression may aid in determining if LH contributes to any of the essential components of cancer such as self-sufficiency in growth signals, evasion of apoptosis, bypassing growth inhibitors, sustained angiogenesis, activation of metastasis and invasion, indefinite replication, evasion of immune destruction, and altered metabolism (Hanahan & Weinberg, 2011).

There is also considerable interest in developing diagnostic biomarkers for ovarian and other cancers at early stages. We have explored a novel biomarker-search paradigm through effective combination of computational and experimental techniques to enhance biomarker discovery from the rather low-yield trial-and-error methods in current use. Our paradigm involves analysis of gene expression data for identification of differentially expressed genes in cancer *versus* controls in conjunction with prediction of proteins that can be secreted into blood and then excreted into urine. The results obtained with primary tumor tissues from patients with gastric cancer, the number two cancer killer in the world, have demonstrated the success of this approach (Cui et al., 2008, 2011a; Hong et al., 2011). In addition, considerable effort has also been invested into the use of gene expression profiling techniques by others to elucidate their utility in predicting metastasis and survivability in ovarian cancer (Lancaster et al., 2006; Sabatier et al., 2009).

Herein we focus our discussion on the *in vitro* use of an ovarian carcinoma cell line, SKOV-3 cells (Warrenfeltz et al., 2008; Puett et al., 2010; Cui et al., 2011b,c), rather than tumor tissues, in part because of the desire to work with an established cell line in which continuing studies can be performed in a controlled and reproducible manner. Such cell lines have provided an enormous wealth of information on the characteristics of ovarian cells and their responses to various inhibitors or growth factors (Choi et al., 2007; Leung & Choi, 2007; Mandai et al., 2007). The question being addressed is whether transcriptomic profiling can be used to determine if LH, acting on LHR+ cells, is stimulatory, inhibitory, or has no effect on ovarian carcinoma cells. Hence, the experimental design has been focused on LH-mediated effects on cancer cells, not if LH is involved in the transformation process of an epithelial-to-carcinoma cell. Due to a considerable interest in the development of reliable serum or urine biomarkers for early detection of ovarian cancer, the results of earlier work are also mentioned. This chapter provides a summary of those studies and links them to other related findings.

2. Properties of the SKOV-3 ovarian carcinoma cell line

The SKOV-3 human ovarian cancer cell line was selected since it does not express LHR (Parrott et al., 2001; Mandai et al., 2007; Warrenfeltz et al., 2008) and can serve as a negative

control. Some lines do, however, appear to express LHR and respond to LH (Lau et al., 2010). Following transfection with a full-length human LHR-pcDNA3 or an empty vector, two sub-lines were generated, one expressing about 12,000 receptors per cell (LHR+) and the other, i.e. the mock-transfected cells, that does not express LHR (LHR-) (Warrenfeltz et al., 2008).

The LHR+ cells bound radio-labeled human chorionic gonadotropin with a Kd of 0.3 nM in saturation binding assays and an IC50 of 0.8 nM in competition binding assays. The expressed LHR was functional as determined by increased production of cyclic AMP and inositol phosphates in response to LH. The LHR- cells, in contrast, exhibited no specific binding of human chorionic gonadotropin and showed no response to LH in terms of second messengers.

Expression of LHR in the SKOV-3 cells, in the absence of LH, had no effect on cell migration or proliferation. It did, however, reduce the invasive index of the cells by a small margin. LH was found to reduce migration and proliferation of LHR+ cells but not of LHR- cells, while the invasiveness was not altered (Warrenfeltz et al., 2008).

3. The transcriptomic profile of SKOV-3 Cells: Alterations associated with LHR expression and activation

RNA was extracted from SKOV-3 cells (Warrenfeltz et al., 2008), and the resulting cDNAs were analyzed by Almac Diagnostics (Durham, NC, USA) using the Affymetrix Human U133 Plus2 Arrays (Cui et al., 2011c). In a parallel study on microRNA expression, the ovarian cancer DSA™ array (Almac Diagnostics) was used (Cui et al., 2011b). The advantages in using ovarian cancer-specific arrays include the gathering of extensive amounts of novel mRNA data that are not covered by other platforms and putting both microRNA and mRNA probes on the same chips, hence avoiding potential noise introduced during data collection on separate chips. Gene expression profiling was done on both the LHR- and the LHR+ cells, and gene-expression data were also collected on the latter at multiple time points, specifically at 1, 4, 8, and 20 h after incubation with human LH. qRT-PCR was carried out to validate a few significantly altered gene expression patterns detected through microarray data analyses (Cui et al., 2011c).

4. Altered gene expression and pathways associated with LHR expression and activation

Among the ~100,000 transcripts profiled in this study, 2,210 and 4,297 were found to show up-regulation and down-regulation with at least 2-fold changes between the LHR+ SKOV3 cells and the control cells, respectively. Most of these differentially expressed transcripts are involved in cell division and in DNA replication and transcription, while genes primarily involved in carbohydrate transport/metabolism and lipid metabolism, cell communication, and ECM interaction were only down-regulated.

When the cells were exposed to LH, 14,903 transcripts exhibited elevated expression, which extend the above functions to include posttranslational modification, RNA processing and modification, intracellular trafficking and secretion, signal transduction mechanisms, and coenzyme metabolism, while 10,389 transcripts found to be down-regulated were associated with cellular defense mechanisms based on our enrichment analyses against COG functions (Tatusov et al., 2000).

In total, 2,373 genes were differentially expressed in LHR+ cells (in the absence of LH) *versus* control (LHR- cells) or LH-treated LHR+ cells. Of these, 689 genes are cancer relevant and 265 are highly expressed in the ovary (GeneGo, 2000). These genes participate in pathways involved in the cell cycle, focal adhesion, cytokine-cytokine receptor interaction, regulation of the actin cytoskeleton, purine metabolism, and the key signaling pathways involved in cell growth, e.g. MAPK, TGF-β, p53, and Jak-STAT. Functional analysis revealed that five major families, namely growth factors, translation regulators, transporters, GPCRs, and ligand-dependent nuclear receptors, were significantly enriched (Fig. 1).

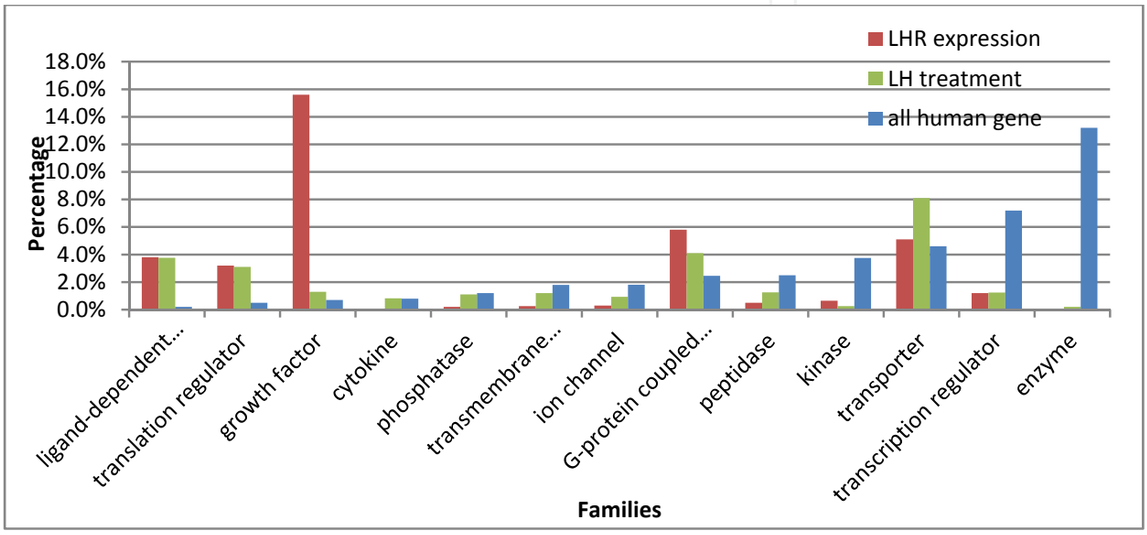
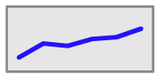
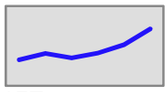
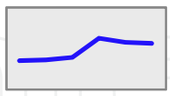
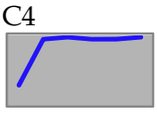
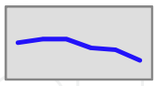
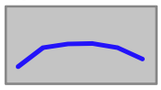
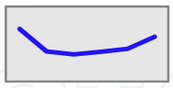
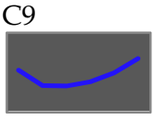


Fig. 1. Distribution of the 2,373 differentially expressed genes in SKOV-3 cells across 13 major functional families. Each red bar represents the percentage of differentially expressed genes associated with LHR expression; each green bar represents the percentage of differentially expressed genes upon incubation with LH; each blue bar is the percentage of all human genes. The y-axis represents the percentage; the x-axis denotes functional families.

Twelve gene clusters, each containing a highly co-expressed pattern, were identified among the 2,373 genes through a clustering analysis, termed a self-organizing map (SOM) (Kohonen 1982) (Table 1). On each gene cluster, Gene Ontology (GO) and pathway enrichment analyses were conducted to identify functional groups and cellular processes that are possibly affected by LHR expression and activation. The details of the findings are discussed elsewhere (Cui et al., 2011c), but the major observations are as follows: LHR expression in control SKOV-3 cells seems to have a positive impact on the activation of gap junctions and the associated growth signaling pathways, and to have moderately suppressed apoptosis, DNA mismatch repair, and the Ras-independent pathways in NK cell-mediated cytotoxicity, which are overall advantageous to cell growth; LH, subsequently, regulated expression of genes involved in the cell cycle, p53 and VEGF signaling, gap junction pathways, immune responses, and the complement and coagulation cascades, as well as a few metabolic pathways (Cui et al., 2011c).

Earlier *in vitro* studies demonstrated that LHR expression, in the absence of LH, slightly inhibited invasiveness, but had no effect on cell proliferation or migration. The addition of LH reduced the growth rate and migratory properties, but was without effect on invasiveness (Warrenfeltz et al., 2008). The current transcriptomic analysis shows that the observed expression changes in the above-mentioned pathways support the previous observations about the measured cellular properties (Warrenfeltz et al., 2008; Cui et al., 2011c).

LHR activated genes		
	Clusters LHR-,LHR+,LH 1-20h (# of genes)	**Enriched GO functions/pathways (P-value)**
LH(↑)	C1  144	extracellular matrix structural constituent 6.54E-04 platelet-derived growth factor alpha-receptor activity 4.38E-03 regulation of vesicle fusion 4.38E-03 hydroxyacid-oxoacid transhydrogenase activity 4.38E-03 pathways: TCR 9.66E-0.2; EGFR1 1.68E-01; IL4 1.79E-01
	C2  157	negative regulation of apoptosis 3.57E-04 leukocyte differentiation 5.75E-04 carboxylic acid metabolic process 7.47E-04 pathways: EGFR1 2.09E-03; TGFBR 4.94E-03; ID 9.55E-03; Kit Receptor 4.23E-02
	C3  152	multicellular organismal development 3.80E-06 cell proliferation 2.31E-05 cyclic-nucleotide phosphodiesterase activity 2.86E-04 regulation of transcription, DNA-dependent 3.44E-04 cell-cell signaling 4.49E-04 pathways: Hematopoietic cell lineage 1.40E-02
LH(-)	C4  205	nervous system development 7.37E-05 neurogenesis 7.84E-04 notch binding 1.71E-03 calcium ion binding 2.16E-03 cell morphogenesis 4.22E-03 pathways: NOTCH 8.29E-02

LHR activated genes		
	Clusters LHR-,LHR+,LH 1-20h (# of genes)	**Enriched GO functions/pathways (P-value)**
LH(↓)	C5  157	response to external stimulus 1.08E-04 positive regulation of cellular metabolic process 1.54E-05 pathways: Androgen Receptor 8.42E-02; EGFR1 2.32E-01
	C6  270	cadmium ion binding 1.70E-06 transcription 2.32E-04 spermidine biosynthetic process 5.52E-04 regulation of RNA metabolic process 7.51E-04 pathways: MT-Heavy Metal-Pathway 1.96E-04; TCR 2.71E-02; IL4 6.76E-02; TNF alpha/NF-kB 9.59E-02
	C7  167	neutrophil chemotaxis 5.52E-06 positive regulation of heart rate 2.03E-05 calcium-mediated signaling 6.13E-05 leukocyte chemotaxis 9.57E-05 regulation of cell migration 2.02E-04 pathways: IL-7 9.19E-02; ID 1.60E-01
LHR suppressed genes		
	Clusters	**Enriched GO functions/pathways (P-value)**
LH(↑)	C8  145	extracellular region 6.34E-11 collagen fibril organization 2.68E-05 complement component C3b binding 3.63e-05 fibrillar collagen 4.56E-05 inflammatory response 9.80E-05 response to external stimulus 3.44E-04 protein digestion 3.98E-04 pathways IL-7 2.84e-03; IL3 3.00E-01; Wnt 3.98e-01
	C9  261	alpha-amylase activity 4.37E-11 amylase activity 2.61E-10 calcium ion binding 5.02E-09 homophilic cell adhesion 3.57E-07 synaptogenesis 3.67E-06 pathway: IL-7 1.16E-01; ID 2.00-01; EGFR1 3.90E-01

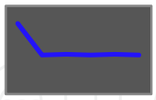
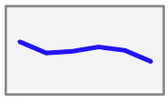
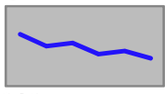
LHR suppressed genes		
	Clusters	**Enriched GO functions/pathways (P-value)**
LH(-)	C10  288	proteinaceous extracellular matrix 6.00E-09 polysaccharide binding 3.04e-08 glycosaminoglycan binding 2.22E-06 regulation of defense response 6.56E-06 G-protein signaling, coupled to IP3 second messenger 1.15E-05 enzyme inhibitor activity 3.59E-05 pathways: Wnt 5.23E-02; EGFR1 1.81E-01
LH(↓)	C11  71	regulation of aldosterone metabolic process 9.16E-06 regulation of hormone metabolic process 5.93E-04 auditory receptor cell differentiation 1.36E-03 epidermis development 1.54E-03 growth factor activity 2.44E-03 pathways: NOTCH 1.58E-02; TGFBR 4.74E-02
	C12  191	cell cycle phase 1.56E-43 mitosis 9.04E43 microtubule cytoskeleton 4.80E-19 pathways: BCR 5.92E-02

Table 1. Enriched GO functions and pathways in each of the 12 gene clusters identified from the differentially expressed genes (modified and extended from Cui et al., 2011c). Plots represent the expression pattern across six groups (LHR- SKOV3 control, LHR expression but with no added LH, and incubation of the LHR+ cells with LH for 1, 4, 8, and 20 h.) "↑" and "↓" denote responses of up-regulation and down-regulation of gene expression, respectively and "-" denotes no alteration of gene expression.

Some of the affected major processes related to cell growth and death (Cui et al., 2011c) have been further investigated. It was found that LHR expression in the control cells led to the up-regulation of genes involved in gap junction, purine metabolism, calcium signaling, and actin cytoskeleton regulation, indicating a possible activation of these processes at a moderate level. In particular, the up-regulation of genes involved in gap junction formation and function may indicate reduced tumor progression and metastasis (Holder et al., 1993). LH-activated genes are involved in VEGF signaling, the Toll-like receptor signaling, and the B-cell receptor signaling pathway, as well as those involved in gap junction and Notch signaling, which may accelerate cell-cell communication and influence several key aspects of normal cell development by regulating differentiation, proliferation, and apoptosis (Sjolund et al., 2005). One particularly interesting observation was that the substantially increased expression of the tumor necrosis factor member 10 gene (*TNFSF10*), involved in natural killer cell-mediated cytotoxicity, may induce apoptosis (Pan et al., 1997). LH also led to

considerable activation of the genes for interleukin-6 (*IL-6*) and IL-8, pleiotropic cytokines, which are believed to be involved in ovarian carcinogenesis and angiogenesis (Asschert et al., 1999; Schwartz et al., 2001; Chou et al., 2005).

5. Comparative profiling between SKOV-3 cells and normal Human Ovarian Surface Epithelium (HOSE) cells, and molecular markers

The genes most highly expressed (top 5%) in SKOV-3 cells are largely different from those in the normal HOSE cells. It was found that 1,056 such genes were specific to the LHR- SKOV-3 cells, involved in regulation of translation, cell division, chromosome partitioning, post-translational modifications, protein turnover, chaperones, and signal transduction mechanisms, suggesting increased cell growth and proliferation. Another 689 genes were specific to the LH-treated cells and found to be associated with coenzyme metabolism, post-translational modifications, nucleotide transport, DNA replication and repair, intracellular trafficking, and secretion.

Two lists of genes, one with 185 up-regulated genes and the other with 248 down-regulated genes, were identified to show differential expressions between normal and cancer cells regardless of LHR expression or activation (Cui et al., 2011c). Functional analyses revealed that the up-regulated genes are involved in cell-cell communication, ECM-receptor interaction, focal adhesion, cell division and chromosome partitioning, as well as carbohydrate transport and metabolism, which are essential to cancer growth. Of interest, 106 of these genes were found to be specific to ovarian cancer, based on our analyses of their differential expression patterns in ovarian cancer *versus* those of other human diseases with available genome-scale expression data in the public database (http://bioinfosrv1.bmb.uga.edu/DMarker/).

These results engender confidence in proposing some of them as potential molecular markers for ovarian epithelial carcinoma cells *versus* normal HOSE cells. Using a prediction method that we recently developed and validated (Cui et al., 2008), 103 of these genes were predicted to have their protein products secreted into circulation, thus providing another important pool of potential serum markers for ovarian cancer (Cui et al., 2011c).

6. Known therapeutic targets involved in the cellular response to LH action

As of now, 39 genes have been proposed as therapeutic targets for ovarian cancer based on our database search against the Therapeutic Target Database (TTD) (Mandai et al., 2007). Among them, endothelin-1 (ET-1), stromal cell-derived factor 1 (SCD-1), and insulin-like growth factor II (IGF2) show the most significant expression changes associated with LH.

ET-1, acting through its receptor, ETAR, has been extensively studied in its physiological roles in vasoconstriction and proliferation of smooth muscle cells, as well as its pathophysiological role in hypertension, heart failure, and coronary vasospasms. Recently it was also identified as important in ovarian cancer initiation and progression (Bagnato & Rosano, 2008; Bhalla et al., 2009; Rosano et al., 2010). These findings have led to the development of endothelin-converting enzyme-1 inhibitors and small interfering RNAs as new therapeutic agents for ovarian cancer (Rayhman et al., 2008). LH increases gene expression of ET-1 by some 10-fold, peaking at 1 h, an observation documented by qRT-PCR

(Cui et al., 2011c). This observation, along with findings that ET_AR shows a moderate elevation in expression, while endothelin-converting enzyme-1 and the endothelin B receptor show no changes in their expression, may indicate a possible enhancement of cell proliferation in response to LH.
SCD-1 has been reported to increase the invasiveness and migration of breast cancer cells (Kang et al., 2005), and IGF2 is known to be a fetal promoter of cell proliferation in various cancers (Zaina et al., 2002). The up-regulation of these genes may suggest that LH exerts positive effects on tumor growth and metastasis. However, reduced cell growth is manifested in LH-treated cells (Warrenfeltz et al., 2008), and thus expression of the negative regulators, e.g. *c-JUN, TNFSF10,* and *MMPs* (Cui et al., 2011c), must assume a dominant role in relating gene expression and tumor cell properties.

7. MicroRNA regulation involved in LH treatment of LHR+ SKOV3 cells

In addition to the aforementioned ~100,000 protein-encoding transcripts, 132 microRNAs were selectively profiled on the DSA array. It is known that small non-coding RNAs serve in various regulatory roles in degradation of mRNAs and inhibition of translation (Bartel, 2004) in all major cellular processes, such as differentiation, apoptosis, and proliferation (Ambros, 2004; Bartel, 2004). Many microRNAs are androgen related, and their deregulation is highly correlated with initiation, progression, and prognosis of cancer (Calin et al., 2005; Yanaihara et al., 2006; Bloomston et al., 2007; Ambs et al., 2008; Garzon et al., 2008; Schetter et al., 2008; Croce, 2009; Wyman et al., 2009).
Recently, 17 microRNAs were found to be differentially expressed in LHR+ SKOV-3 cells *versus* control cells (Cui et al., 2011b), specifically: six up-regulated (miR-101-1, -101-2, -199b, -559, -573, and -7-3) and 11 down-regulated (miR-103-2, -200c, 151, 29c, 301b, 548a2, 552, 561, 566, 613, and 642). After incubation with LH, 57 microRNAs were found to be differentially expressed, including the most highly-expressed microRNAs, such as miR-21, -200c, -593, -103-1, and -124-3. Some of these microRNAs are located in the fragile sites (also called *hot spots*) in the human genome (Calin et al., 2004; Bignell et al., 2010), where genomic alternations in these regions were found to be associated with certain types of cancer. For example, the loss of 11p15 (covering miR-210) is found in ovarian cancer (Peng et al., 2000), and amplification of 17q23 (covering miR-301a and miR-21) is found in breast cancer (Barlund et al., 2000), as well as those reported (Calin et al., 2002; He et al., 2005).
The present focus is to examine LH-mediated transcriptional changes of the microRNAs, but it should be mentioned that the SKOV-3 cancer cells have probably undergone some genomic alternations, resulting in altered gene expression levels in the control cancer cells that cannot be determined by our current expression data. Collectively, 65 microRNAs have been identified to be differentially expressed in LHR+ SKOV-3 cells *versus* control. The mRNAs with which the microRNA may interact have been studied in order to infer potentially regulated processes involving the microRNAs (Cui et al., 2011b).
Specifically, Spearman correlation analysis was performed between the expression of the 65 differentially-expressed microRNAs and the expression of 60,860 well-annotated mRNAs across all sample groups under consideration, resulting in 62,150 and 931,009 microRNA-mRNA pairs with significantly correlated expressions, positive or negative, using cutoffs $|\text{rho}|>0.8$ and P-value <0.05, where rho represents the Spearman correlation coefficient. More positively correlated microRNA-mRNA pairs than negatively correlated pairs were found for the vast majority of the microRNAs, except for nine microRNAs: miR-181B2, miR-

582, miR-497, miR-559, miR-561, miR-101-1, miR-187, miR-572, and miR-301A. The prevalence in positively correlated microRNA-mRNA pairs suggests that such microRNAs are probably located in introns or 5′ untranslated regions of the corresponding mRNAs and hence are regulated by the same transcription regulators of their host genes. The negatively correlated pairs may indicate possible biochemically important interactions.

MiRanda (Miranda et al., 2006) and TargetScan (Lewis et al., 2005) were applied for microRNA target prediction. A total of 584 genes were predicted to interact with the 65 differentially expressed microRNAs. Although not all predicted pairs possess high correlations, it does show a trend that the percentage of predicted pairs decreased as the coefficients increase along its distribution. With the above cutoffs, only 155 genes were retained as highly-confident microRNA targets for further analysis. For each of the 65 microRNAs under consideration, its function was predicted through identification of the functions and pathways enriched by their target genes; the functions of 16 differentially-expressed microRNAs were so predicted (Cui et al., 2011b).

For example, miR-199b-5p is predicted to participate in angiogenesis, nucleotide excision repair, the PDGF signaling pathway, the cadherin/Wnt/integrin signaling pathway, apoptosis, and the MAPK signaling pathway. MiR-101 is predicted to be involved in the Wnt/MAPK/cadherin signaling pathway, as well as in hypertrophic cardiomyopathy, melanogenesis, the metabotropic glutamate receptor group III pathway, and ubiquitin-mediated proteolysis. In addition, it may also be involved in the regulation of the mRNAs involved in cyclic AMP regulation; cyclic AMP-specific phosphodiesterase 4D (*PDE4D*) was highly up-regulated by LH in LHR+ cells (Cui et al., 2011c). MiR-29c is predicted to regulate ECM-receptor interaction, focal adhesion, collagen α chains, and the integrin signaling pathway. It is noteworthy that several of the microRNAs are predicted to be potentially involved in regulation of various tyrosine and serine/threonine kinases (Cui et al., 2011b). The main regulation of miR-129 is that of angiogenesis, the Wnt signaling pathway, transcription regulation, and cell junction. The predicted involvement of miR-199b-5p, miR-101, and miR-129 in the Wnt pathway may suggest its possible role in ovarian tumorigenesis (Gatcliffe et al., 2008).

To affirm that some microRNAs participate in the LH regulation of cancer cells, the experimentally validated targets of the 65 differentially-expressed microRNAs were examined, and 70 such genes were extracted from miRecords (Xiao et al., 2009). Of these, 20 genes are differentially expressed in the LH-treated cells, and some are known to be involved in the regulation of cell migration and proliferation (IRS1, IRS2, IL6R, TPM1, GLI1, BMPR2, GRN), cell surface receptor-linked signal transduction (SOCS5, RAF1), anti-apoptosis (FAS, MCL1, SGK3), and transcription regulation (DNMT3B, GLI1, EZH2) (Cui et al., 2011b). Only six of the 20 genes exhibited highly correlated expression patterns with some of the 65 microRNAs (Cui et al., 2011c), namely IRS1, IRS2, and RAF1 with miR-7-1, SGK3 and MTAP with miR-21, and GRN with miR-659. The expression changes of these genes indicate that LH may impose a positive regulation of cell proliferation, nucleotide metabolic processes, and cell surface receptor-linked signal transduction, and a negative regulation of apoptosis on ovarian cancer cells through these microRNAs.

Additionally, 54 oncogene and tumor-suppressor genes (Jiang et al., 2009; Bignell et al., 2010) were examined to determine if some of the microRNAs may participate in transcriptional regulation (Cui et al., 2011b). It was found that miR-21 was up-regulated while its target, TPM1, a tumor suppressor gene, was down-regulated in response to LH, suggesting a role of miR-21 in inhibiting apoptosis and subsequently having a positive

impact of LH on cancer development. However, our other observations such as up-regulation of *NF1*, *RB1*, and *SUFU* may indicate a negative effect on cancer growth, consistent with our previous report (Warrenfeltz et al., 2008).

As important gene regulators, microRNAs exhibit characteristics that allow speculation on some of the key roles they may have in regulating the downstream LHR signaling in ovarian cancer. The indicative clues of regulating apoptosis and cell proliferation may provide useful guidance for further research on the causes and treatment of ovarian cancer. It should be noted from the above analysis that detection of microRNA-mRNA pairs is a key step to understand the functions of microRNAs. All current computational programs used for predicting microRNA/mRNA interactions are mainly based on information embodied in the sequence and structure (Dai & Zhou, 2010). Yet the Argonaute (Ago) proteins have a central role in recognizing and binding to target mRNAs (Wang et al., 2010), and we thus anticipate that a method taking into consideration the sequence or structure information of AGO proteins may efficiently improve the prediction performance.

8. Conclusions

Numerous studies have appeared on the physiological roles and biochemical mechanisms of the pituitary gonadotropins, LH and FSH, in developmental and reproductive processes, as well as the pathophysiology associated with aberrant expression and mutations in the genes encoding the three gonadotropin subunits and the two gonadotropin receptors. Recent experimental findings and epidemiological evidence has arisen suggesting that the hormones and receptors are also involved in the development and/or progression of ovarian cancer. There is, however, much controversy associated with the role(s), if any, of the gonadotropins in ovarian cancer. Research undertaken in our laboratory has focused on experimental measurements of altered cellular properties and transcriptomic profiling of SKOV-3 cells in response to LH, in an effort to clarify aspects of this important area (Warrenfeltz et al., 2008; Puett et al., 2010; Cui et al., 2011a,b,c). This work has established that the expression of many genes and microRNAs is altered by LHR expression in SKOV-3 cells and by activation of the receptor with its natural ligand, LH. Some of the changes involve genes and pathways associated with cell growth, apoptosis, and many more cellular processes. Of interest was the observation that many genes had altered expression patterns upon expression of LHR in the absence of ligand. Such changes presumably arise from ligand-independent actions of LHR and from the unoccupied receptor adopting an active, or active-like, conformation periodically. Incubation of the cells with LH resulted in the expression of myriad genes (over 2,000), many of which are important in biological processes such as proliferation, apoptosis, and others. Further studies on microRNAs identified 65 that exhibited altered expression in LHR+ cells and LHR+ cells incubated with LH. Some of these microRNAs may aid in diminishing cell proliferation and possibly enhancing apoptosis. The conflicting results often obtained with transcriptomic profiling, including evidence for both positive and negative enhancement of important processes such as proliferation, apoptosis, etc., documents the need to always have experimental studies on the cellular phenotype with regard, in the present case, to LHR expression and LH-mediated LHR activation. The net effect, as determined from cell studies is a slight inhibition of proliferation, invasiveness, and migration upon LHR expression and activation. Of potential importance was the observation that some 100 genes were identified that may lead to secreted proteins, thus offering an array of possible serum

biomarkers specific for ovarian cancers expressing LHR in the presence of circulating LH, often the case in post-menopausal women.

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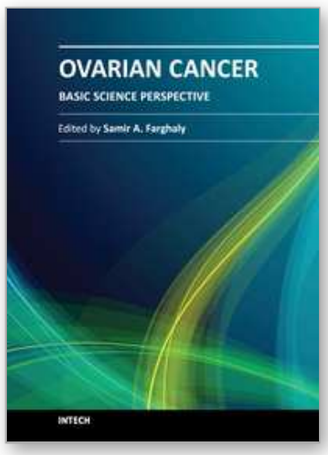

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Worldwide, Ovarian carcinoma continues to be responsible for more deaths than all other gynecologic malignancies combined. International leaders in the field address the critical biologic and basic science issues relevant to the disease. The book details the molecular biological aspects of ovarian cancer. It provides molecular biology techniques of understanding this cancer. The techniques are designed to determine tumor genetics, expression, and protein function, and to elucidate the genetic mechanisms by which gene and immunotherapies may be perfected. It provides an analysis of current research into aspects of malignant transformation, growth control, and metastasis. A comprehensive spectrum of topics is covered providing up to date information on scientific discoveries and management considerations.

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