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Adiposity Alters Genes Important in Inflammation and Cell Cycle Division in Human Cumulus Granulosa Cell

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Abstract

Objective: To determine whether obesity alters genes important in cellular growth and inflammation in human cumulus granulosa cells (GCs). **Methods:** Eight reproductive-aged women who underwent controlled ovarian hyperstimulation followed by oocyte retrieval for in vitro fertilization were enrolled. Cumulus GC RNA was extracted and processed for microarray analysis on Affymetrix Human Genome U133 Plus 2.0 chips. Gene expression data were validated on GCs from additional biologically similar samples using quantitative real-time polymerase chain reaction (RT-PCR). Comparison in gene expression was made between women with body mass index (BMI) <25 kg/m^2 (group 1; n = 4) and those with BMI $\geq$25 kg/m^2 (group 2; n = 4). **Results:** Groups 1 and 2 had significantly different BMI (21.4 $\pm$ 1.4 vs 30.4 $\pm$ 2.7 kg/m^2, respectively; $P = .02$) but did not differ in age (30.5 $\pm$ 1.7 vs 32.7 $\pm$ 0.3 years, respectively; $P = .3$). Comparative analysis of gene expression profiles by supervised clustering between group 1 versus group 2 resulted in the selection of 7 differentially expressed genes: fibroblast growth factor 12 (FGF-12), protein phosphatase 1-like (PPM1L), zinc finger protein multitype 2 (ZFPM2), forkhead box M1 (FOXM1), cell division cycle 20 (CDC20), interleukin 1 receptor-like 1 (IL1RL1), and growth arrest-specific protein 7 (GAS7). FOXM1, CDC20, and GAS7 were downregulated while FGF-12 and PPM1L were upregulated in group 2 when compared to group 1. Validation with RT-PCR confirmed the microarray data except for ZFPM2 and IL1RL. As BMI increased, expression of FOXM1 significantly decreased ($r = -.60$, $P = .048$). **Conclusions:** Adiposity is associated with changes in the expression of genes important in cellular growth, cell cycle progression, and inflammation. The upregulation of the metabolic regulator gene PPM1L suggests that adiposity induces an abnormal metabolic follicular environment, potentially altering folliculogenesis and oocyte quality.

Keywords

obesity, granulosa cell, cumulus, inflammation, cell cycle

Introduction

Obesity continues to be an epidemic in the United States and throughout the world with recent reports indicating that approximately 38% of women are either overweight or obese in 2013.[1,2] In reproductive-aged women, contributions of obesity to poor reproductive outcomes are well recognized.[3] For instance, obesity is associated with impaired response to ovarian stimulation during in vitro fertilization (IVF), lower mature oocyte yield, lower embryo implantation rates, and significantly lower live births after IVF.[4] Despite these findings, other studies did not report poorer embryo quality in obese women, thus pointing to the endometrium as another main player in the impaired reproductive outcome of obese patients.[5,6] Additionally, poor IVF outcomes associated with obesity have been attributed to changes in endometrial gene expression at the time of implantation.[7] Obesity is also associated with ovarian intrafollicular alterations at multiple cellular levels including steroidogenic, metabolic, and inflammatory pathways.[8] We have previously shown that obese women have suboptimal corpus luteum function.[9,10] Additionally, we[11] and others[12,13]

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have shown that obese women have lower ovarian reserve than normal weight women; as reflected by their low serum anti-Mullerian hormone (AMH) levels and diminished AMH gene expression in their granulosa cells (GCs).[14]

Oocyte development is governed by paracrine interactions with GCs by which these cells modulate the growth and development of oocyte and vice versa.[15] This bidirectional crosstalk between GCs and the oocyte affects the hormonal production and the expression of genes associated with follicular development. Hence, the growth, development, and remarkable functional differentiation of GCs are one of the significant events that are required for follicle maturation.[16,17] Abnormal expression of those developmentally related genes could then lead to cellular communication dysfunction and dysregulation of normal follicle recruitment and development. Expression of GATA4, for instance, occurs in potentially mitotic and proliferating GCs, but expression is lost once these cells become either terminally differentiated (from granulosa to luteal cells) or apoptotic during follicular atresia.[18] Data demonstrated that there are many genes that are capable of increasing susceptibility to metabolic disease traits such as obesity, diabetes, and atherosclerosis.[19] Such genes include lipoprotein lipase (*Lpl*), lactamase β (*Lactb*), and protein phosphatase 1-like (*Ppm1l*).[19] Additionally, perturbation of genes such as Gpx3 has been implicated in the increase in inflammatory signals and oxidative stress and hence obesity-related metabolic disorders.[20]

There are 2 types of GCs, cumulus and mural. It is well established that cumulus GCs reflect the health of the oocyte more than mural GCs do.[21,22] Little is known about the effect of obesity, a known inflammatory state,[23] on ovarian physiology and in particular on cumulus GC function, specifically cell cycle and cellular growth. Thus, we herein hypothesize that GC dysfunction contributes to abnormal follicular maturation and subsequent corpus luteum dysfunction in obese women, and that overweight and obese women have altered GC gene expression compared to normal weight women.

Materials and Methods

Patients

Eight infertile women undergoing fresh IVF and intracytoplasmic sperm injection (ICSI) cycles utilizing autologous oocytes at the University of Vermont College of Medicine (UVM) between August 2012 and June 2013 were prospectively enrolled. Inclusion criteria consisted of women with normal ovarian reserve; defined as day 3 follicle-stimulating hormone (FSH) <10 mIU/mL and day 3 estradiol (E2) <80 pg/mL. Reasons for infertility were male, tubal, unexplained infertility, and oocyte donation. Women with polycystic ovarian syndrome as defined by Rotterdam criteria[24] were excluded from the study. The following characteristics of participants and their IVF cycle parameters were collected: age, body mass index (BMI), serum day 3 FSH level, antral follicle count by ultrasound, total days of stimulation, total dose of gonadotropins used during controlled ovarian hyperstimulation, peak serum E_2 level on day of human chorionic gonadotropin (hCG), and total number of oocytes retrieved. Participants had no health comorbidities, and they were on no medications other than IVF medications. All patients gave informed consent, and the study was approved by the institutional review board of UVM. Comparisons were made between women with BMI <25 kg/m^2 (group 1; n = 4) and BMI ≥25 kg/m^2 (group 2; n = 4).

Collection of GCs

The IVF/ICSI cycles using either gonadotropin-releasing hormone (GnRH) agonists or GnRH antagonists for hypothalamic pituitary suppression were used. For controlled ovarian hyperstimulation, injectable gonadotropins were used to stimulate follicular growth, which was monitored with transvaginal ultrasounds and serum estradiol (E_2) levels. Human chorionic gonadotropin injection (10 000 U hCG [Novarel; Ferring Pharmaceuticals, Parsippany, New Jersey] or recombinant hCG 250 μg [Ovidrel; EMD Serono, Rockland, Massachusetts]) was administered when at least 2 follicles had a mean diameter greater than 17 mm. Oocyte retrieval was performed 34 hours after hCG injection under transvaginal ultrasound guidance.

Follicular aspirates included oocytes surrounded by cumulus GCs. After identification of the cumulus–oocyte complex in the aspirate, cumulus GCs were collected by mechanically excising the cumulus cell layer from each oocyte then washing with PBS. For each patient, cumulus GCs were pooled to extract enough RNA to allow their quantification and to study gene expression.

RNA Extraction

RNA was isolated from the collected cumulus GCs using phenol–chloroform extraction (Trizol reagent; Invitrogen, Carlsbad, California) according to the manufacturer's instructions, as previously described.[14,25] Total RNA was precipitated with propanol, washed with 75% ethanol, air dried, and reconstituted in diethylpyrocarbonate-treated water. The RNA was stored at −20°C until further analysis.

Microarray Analysis

Each of the 4 participants in each group was submitted to a unique microarray. Labeled complementary DNA (cRNA) probes were generated from total RNA samples using the MessageAmp Premier RNA Amplification Kit (Ambion, Grand Island, New York). Briefly, the procedure consists of reverse transcription of 300 ng of total RNA with a T7 oligo (dT) primer bearing a T7 promoter sequence followed by in vitro transcription of the resulting DNA with T7 RNA polymerase to generate antisense RNA copies of each mRNA. The quality of total RNA and labeled cRNA was assessed with Agilent's Lab-on-a-Chip total RNA nano-biosizing assay (Agilent Technologies, Palo Alto, California). Biotinylated cRNA probes were hybridized to Human Genome U133 Plus 2.0 chips. The chips were washed and stained using the Affymetrix Fluidics

Table 1. Primers Used in the Study.

Gene		Sequence Primers (5′-3′)
FGF12	Forward	CTGTAGAGATAGCCTTCACCATTC
	Reverse	CAAGGACGAAAACAGCGACTA
PPM1L	Forward	GACCACGTTGAGATTTTTCAGC
	Reverse	TCACAAGCCTTACCAGTTGAAG
ZFPM2	Forward	GAAGTTGCTGTCGACTCTGAA
	Reverse	ACACACAGTCAGAGAAACCG
CDC20	Forward	GCTGTAGAGTACTTTCAGTCTGT
	Reverse	GCCCACCAAGAAGGAACA
IL1RL1	Forward	TTTCCAGGCCCCATGATTG
	Reverse	ACAACGAGTTACCAATACTTGCT
GAS7	Forward	GGCAGAACTTGAGGTGAAC
	Reverse	CTCAGAACTCCTTGGCTTCAC
FOXM1	Forward	GTGAATGGTCCAGAAGGAGAC
	Reverse	ACCACTTTCCCTACTTTAAGCAC

Abbreviations: CDC20, cell division cycle 20; FGF12, fibroblast growth factor 12; FOXM1, forkhead box; GAS7, growth arrest-specific protein 7; IL1RL1, interleukin 1 receptor-like 1; PPM1L, protein phosphatase 1 like; ZFPM2, zinc finger protein multitype 2.

Station 400 according to the manufacturer's standard protocol. The chips were scanned using the Affymetrix GeneChip Scanner 3000 (Affymetrix, Santa Clara, California). The image data on each individual microarray chip was scaled to 500 target intensity, using the Microarray Suite software (Affymetrix).

Validation of our discovery set and prediction capabilities were performed using BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.html), an open-source array analytical platform developed by the National Cancer Institute under the direction of Dr Richard Simon. BRB-ArrayTools uses an internal algorithm to summarize the results from at least 6 different classifier methods to produce the highly represented gene list for the experimental design. BRB-ArrayTools can perform complete cross-validation estimation with a doubly nested cross-validation procedure, where the outer loop estimates the prediction error for the test with 1 or more samples removed from the training set, and the inner loop would fine tune the threshold for the univariate test. Again, candidates for our classifiers will be decided based on statistical significance between categories, correlative copy number alterations, and bioinformatic evidence.

Reverse Transcription and Real-Time Polymerase Chain Reaction

The RNA was cleaned using the RNeasy mini kit (Qiagen, Valencia, California), and reverse transcription was performed using Superscript III reverse transcriptase (Invitrogen). RNA quality analysis was performed using the Nanodrop Spectrophotometer and Agilent Bioanalyzer (Santa Clara, California). Samples with a minimum concentration of 10 ng/μL and with an A 260/280 ratio of 1.8 to 2.0 were used. Evaluation of mRNA expression levels in GCs was achieved by real-time polymerase chain reaction (RT-PCR) kinetics using the SYBR Green I chemistry as described elsewhere.[14,25-27] The cDNA was then amplified in triplicate using LightCycler 480 SYBR Green PCR Master Mix (Roche Life Sciences, Indianapolis, Indiana). The primers used (Table 1) were synthesized by Fisher Scientific (Pittsburg, Pennsylvania). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control, and the levels of mRNA for each gene relative to GAPDH were calculated using the $2^{-\Delta\Delta CT}$ method.[28]

Statistics

Comparisons between group 1 and group 2 were performed using unpaired t test. Among all participants, linear regression was performed between each participant's BMI and each gene's geometric mean of intensity by microarray. Demographic and clinical data were expressed as mean $\pm$ standard error of the mean. $P < .05$ was considered statistically significant.

Results

Groups 1 and 2 had, as expected, significantly different BMI (21.4 ± 1.4 vs 30.4 ± 2.7 kg/m^2, respectively; $P = .02$) but did not differ in age (30.5 ± 1.7 vs 32.7 ± 0.3 years, respectively; $P = .3$), day 3 FSH (8.1 ± 0.8 vs 6.2 ± 0.5, respectively; $P = .1$), antral follicle count (16 ± 3.5 vs 24.5 ± 7.2, respectively; $P = .4$), total days of stimulation (9.7 ± 0.8 vs 10.5 ± 0.9, respectively; $P = .6$), total dose of gonadotropins (2813.0 ± 584.6 vs 3338.0 ± 856.0 IUs, respectively; $P = .6$), serum E_2 on day of hCG (3291 ± 847 vs 2279 ± 394, respectively; $P = .3$), or total number of oocytes retrieved (16.5 ± 4.6 vs 12.8 ± 2.4, respectively; $P = .5$). Comparative analysis of gene expression profiles (Supplemental Table 1) by supervised clustering between group 1 versus group 2 resulted in statistically significant changes in 107 genes, of which 7 were selected for validation: FGF-12, PPM1L, ZFPM2, forkhead box M1 (FOXM1), CDC20, IL1RL, and GAS7. These genes were chosen based on their published data pertaining to their roles in ovarian function, fertility, GC function, adipocyte proliferation, and obesity in other systems.[18-20,29-33]

The FOXM1, CDC20, and GAS7 were downregulated ($P < .05$; fold change >1.5) while FGF-12 and PPM1L were upregulated in group 2 when compared to group 1 ($P < .05$; fold change >1.5). Validation with RT-PCR confirmed the microarray data except for ZFPM2 and IL1RL where there was a discrepancy between the microarray and RT-PCR data (Figure 1). Regression analysis was performed between the differentially expressed genes and BMI and the data revealed that as BMI increased, several genes were either up- or downregulated: LARP1, ZNF521, DMKN, TMLHE, ETHE1, and KIAA1324L were positively correlated while C14orf145, HIST1H3C, STMN1, BLM, CTNNBIP1, ATF5, BCL11A, FAM89B, CDKN1C, SMC2, CCNB1, STXBP5L, POC1A, KIFC1, DLGAP5, CEP55, CENPK, HIST3H2A, and FOXM1 were negatively correlated with BMI (Table 2).

Discussion

One of the most significant roles of the ovary is to undergo a continuous folliculogenesis to produce viable and competent

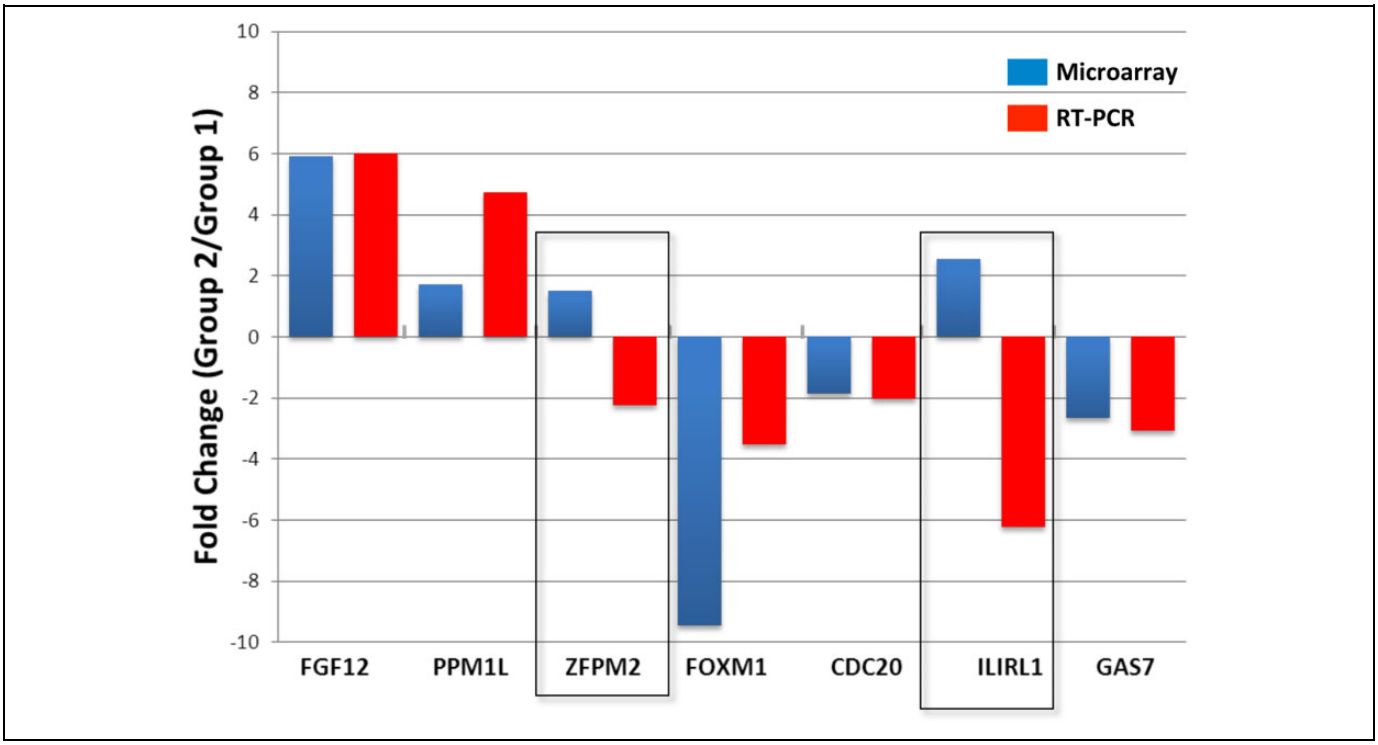

Figure 1. Relative gene expression for selected genes, comparing microarray with quantitative RT-PCR. The blue bars represent the fold change between the mean expression level (RT-PCR, n = 4) and the red bars represent the fold change between the mean expression level (array, n = 4) in cumulus granulosa cells (GCs) between overweight/obese and normal weight participants. RT-PCR indicates real-time polymerase chain reaction. (The color version of this figure is available in the online version at http://rs.sagepub.com/.)

oocytes. Understanding the molecular mechanism of follicular development is essential to unravel the complex synergies orchestrated during the process of forming the fertilizable ovum. Cumulus GCs are required during the major stages of oocyte growth and development including ovulation and fertilization. The objective of this study was to determine the relationship of obesity, a worsening epidemic,[2] to human cumulus GC gene expression. Using microarray and RT-PCR technologies, we have demonstrated that the expression of FOXM1, CDC20, and GAS7 was downregulated, whereas the expression of FGF-12 and PPM1L was upregulated in cumulus GCs of overweight/obese women. Additionally, BMI was correlated with several genes whose function is described in Table 2.

The FGF-12 and PPM1L play an important role in inflammation. In addition to inflammation, FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion.[34] The PPM1L gene has a causal relationship with obesity and metabolic syndrome.[19] The PPM1L gene encodes an endoplasmic reticulum (ER)-targeted protein phosphatase with high specificity for an ER membrane localized kinase/endoribonuclease inositol-requiring protein 1, a key regulator in the ER stress signaling pathway.[35] Our results demonstrated that FGF-12 and PPM1L were upregulated in cumulus GC of obese women providing evidence that inflammation and ER stress pathways are activated in obesity.

The FOXM1, CDC20, and GAS7 genes play a role in cellular growth and division. The FOXM1 is a transcription factor that regulates the expression of multiple cell cycle genes.[36,37] The FOXM1 regulates all phases of the cell cycle; for instance, it can control the G1/S transition by activating the cyclin-dependent kinases, Cdk4, Cdk6, and Cdk2.[36,37] Loss of FOXM1 expression generates mitotic spindle defects, delays cells in mitosis, and induces mitotic catastrophe.[38] Additionally, depletion of FOXM1 generates cells that enter mitosis but are unable to complete cell division, resulting in either mitotic catastrophe or endoreduplication indicating that FOXM1 depletion causes cell death.[38] The CDC20 is an essential regulator of cell division in humans. The CDC20 is one of the most important activators of anaphase promoting complex, and it targets mitotic cyclins and securin for degradation, thereby promoting sister-chromatid separation.[39] High-fat diet has been shown to cause alteration in CDC20 in pulmonary tissue.[40] The GAS7 was originally identified as a gene expressed in serum-starved NIH 3T3 mouse embryonic fibroblast cells, and its protein structure resembles that of Oct2 and synapsins, which are involved in neuronal development and neurotransmitter release, respectively.[41,42] The presence of different isoforms of the GAS7 protein suggests that it may play different roles in adult cells and embryonic stem cells.[42] Moreover, the localization of GAS7 protein to the neuroectodermal cells during the later phases of embryogenesis reveals that these cells regulate expression of GAS7 developmentally and according to the

Table 2. Statistically Significant Correlations Between Differentially Expressed Genes and Body Mass Index.[a]

Gene Symbol	*r* Value	*P* Value	Official Full Name and Function
	Positive values		
LARP1	.75	.007	Name: La ribonucleoprotein domain family, member 1 Functions: • Important regulator of cell growth and proliferation • Stimulates the translation of mRNAs containing a 5′-terminal oligopyrimidine (TOP) motif, encoding for components of the translational machinery
ZNF521	.73	.010	Name: Zinc finger protein 521 Functions: • Stem cell-associated transcription cofactor • Implicated in the control of hematopoietic, osteoadipogenic, and neural progenitor cells
DMKN	.73	.010	Name: Dermokine Functions: • Known to be upregulated in inflammatory diseases • First observed as expressed in the differentiated layers of skin • Involved in the early endosomal trafficking
TMLHE	.72	.011	Name: Trimethyllysine hydroxylase, epsilon Functions: • Encodes the protein trimethyllysine dioxygenase, which is the first enzyme in the carnitine biosynthesis pathway • Carnitine plays an essential role in the transport of activated fatty acids across the inner mitochondrial membrane
ETHE1	.63	.036	Name: Ethylmalonic encephalopathy 1 Functions: • Encodes a sulfur dioxygenase that localizes within the mitochondrial matrix • The enzyme functions in sulfide catabolism
KIAA1324L	.63	.038	Name: KIAA1324-like Functions: • Regulator of the bone morphogenetic protein (BMP) pathway that has a positive role in BMP signaling • Plays an essential role in epidermal differentiation during early embryonic development
	Negative values		
C14orf145 or CEP128	−.74	.009	Name: Centrosomal protein 128 kDa Function: • Component of human centrosomes
HIST1H3C	−.73	.010	Name: Histone cluster 1, H3c Functions: • Encodes a member of the histone H3 family • Transcripts from this gene lack polyA tails but instead contain a palindromic termination element
STMN	−.72	.011	Name: Stathmin 1 Functions: • Encodes a ubiquitous cytosolic phosphoprotein proposed to function as an intracellular relay integrating regulatory signals of the cellular environment • Involved in the regulation of the microtubule filament system by destabilizing microtubules • Prevents assembly and promotes disassembly of microtubules
BLM	−.69	.017	Name: Bloom syndrome, RecQ helicase-like Functions: • Product is related to the RecQ subset of DExH box-containing DNA helicases and has both DNA-stimulated ATPase and ATP-dependent DNA helicase activities • Mutations causing Bloom syndrome delete or alter helicase motifs and may disable the 3′-5′ helicase activity • The normal protein may act to suppress inappropriate recombination
CTNNBIP1	−.68	.020	Name: Catenin, beta interacting protein 1 Function: • The encoded protein is a negative regulator of the Wnt signaling pathway

(continued)

Table 2. (continued)

Gene Symbol	*r* Value	*P* Value	Official Full Name and Function
ATF5	−.67	.023	Name: Activating transcription factor 5 Function: Plays a role in the regulation of osteogenic differentiation in adipose-derived stem cell
BCL11A	−.66	.025	Name: B-cell CLL/lymphoma 11A (zinc finger protein) Functions: • Encodes a C2H2 type zinc-finger protein • The corresponding mouse gene is a common site of retroviral integration in myeloid leukemia and may function as a leukemia disease gene, in part, through its interaction with BCL6 • Possibly involved in lymphoma pathogenesis
FAM89B or LRAP25	−.66	.026	Name: Family with sequence similarity 89, member B Function: • Functional relevance of the LRAP25-MRCK complex in LIMK1-cofilin signaling and the importance of LRAP adaptors as key determinants of MRCK cellular localization and downstream specificities
CDKN1C	−.65	.031	Name: Cyclin-dependent kinase inhibitor 1C Functions: • The encoded protein is a tight-binding, strong inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation • Mutations in this gene are implicated in sporadic cancers and Beckwith-Wiedemann syndrome, suggesting that this gene is a tumor suppressor candidate
SMC2	−.65	.031	Name: Structural maintenance of chromosomes 2 Function: • Critical for mitotic chromosome condensation and DNA repair
CCNB1	−.64	.031	Name: Cyclin B1 Functions: • The protein encoded by this gene is a regulatory protein involved in mitosis • The gene product complexes with p34(cdc2) to form the maturation-promoting factor (MPF)
STXBP5L	−.63	.036	Name: Syntaxin binding protein 5-like Function: • Important component in the neurotransmitter release process
POC1A	−.63	.036	Name: POC1 centriolar protein A Functions: • Plays an important role in basal body and cilia formation • This gene encodes 1 of the 2 POC1 proteins found in humans
KIFC1	−.62	.040	Name: Kinesin family member C1 Functions: • In cancer cells, KIFC1 plays an essential role in bipolar spindle formation by clustering the multiple poles during mitosis • Plays an essential role in maintaining chromosomal stability in mitosis
DLGAP5	−.62	.042	Name: Discs, large (Drosophila) homolog-associated protein 5 Function: • Component of the chromatin-dependent pathway for spindle assembly
CEP55	−.62	.043	Name: Centrosomal protein 55 kDa Function: • Involved in centrosome-dependent cellular functions, such as centrosome duplication and/or cell cycle progression, or in the regulation of cytokinesis
CENPK	−.62	.043	Name: Centromere protein K Functions: • CENPK is a subunit of a CENPH (MIM 605607)-CENPI (MIM 300065)-associated centromeric complex that targets CENPA (MIM 117139) to centromeres • Required for proper kinetochore function and mitotic progression
HIST3H2A	−.61	.044	Name: Histone cluster 3, H2a Functions: • Basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes • Encodes a member of the histone H2A family

(continued)

Table 2. (continued)

Gene Symbol	*r* Value	*P* Value	Official Full Name and Function
FOXM1	−.60	.048	Name: Forkhead box M1 Functions: • The protein encoded by this gene is a transcriptional activator involved in cell proliferation • The encoded protein is phosphorylated in M phase and regulates the expression of several cell cycle genes, such as cyclin B1 and cyclin D1

Abbreviations: ATP, adenosine triphosphate; Limk1, Lim kinase 1; LRAP25, leucine repeat adaptor protein 25; MRCK, myotonic dystrophy kinase-related Cdc42-binding kinase; POC1, protein of centriole 1; mRNA, messenger RNA.
[a]Official full name and function of each gene are summarized.

needs of the embryonic stem cells.[42] Interestingly, GAS7 has been implicated in the regulation of fat metabolism and insulin signaling pathway, indicating a role of this gene in obesity.

One of the mechanisms by which overweight/obese women have alterations in GC gene expression could be via changes in adipokines. Obese women have elevated serum and follicular fluid leptin, and lower serum and follicular fluid adiponectin compared to normal weight women.[43] There is strong evidence for the role of leptin and adiponectin in ovarian physiology.[14,44] Adiponectin has a direct effect on late stages of folliculogenesis, and additive interactions of adiponectin with insulin and gonadotropins in inducing periovulatory changes in ovarian follicles have been demonstrated.[45,46] Ledoux et al[46] demonstrated that adiponectin receptor, AdipoR1 and AdipoR2, mRNAs are present in GCs and adiponectin provokes expression of genes associated with inflammation and periovulatory remodeling of the ovarian follicle: cyclooxygenase 2, prostaglandin E synthase, and vascular endothelial growth factor. Additionally, Chabrolle et al[47] showed that these receptors are detected in human GCs and that adiponectin increases IGF-1-induced estradiol and progesterone secretion in primary human GCs. On the other hand, data have demonstrated that leptin suppresses insulin-like growth factor 1 induced steroid release by human GCs and that leptin suppresses AMH gene expression in human luteinized GCs.[14,47,48] Therefore, alterations in these adipokines in the context of obesity may be, in part, responsible for the alterations in cell cycle and inflammatory genes in GCs.

Limitations to our study include a small sample size. One reason is that we used only high-quality samples with enough extracted RNA for both microarray and RT-PCR. A second limitation to this study is that we used a luteinized GC model, as these cells were collected from women who were hyperstimulated with gonadotropins. However, although luteinized GCs may not be ideal for studying ovarian physiology, this model has been a long-standing effective tool to characterizing ovarian physiology in humans by others and us.[14,25,27,49,50] Another limitation is that we did not evaluate mural GCs in addition to cumulus GCs. The reason why we studied cumulus, rather than mural GCs, is that cumulus cells reflect the health of the oocyte more than mural GCs do.[21,22]

The present study demonstrated that obesity exerts an inflammatory effect in human cumulus GCs and induces disturbances in genes involved in cell cycle division and cellular growth. The upregulation of the metabolic regulator gene PPM1L suggests that adiposity induces an abnormal metabolic microenvironment around the oocyte. Elucidation of mechanisms involved in these responses is essential for the identification of potential biomarkers or the creation of preventive therapies for ovarian dysfunction in obese women.

Authors' Note

Sangita Jindal and Nanette Santoro equally contributed to this work and cosenior authors.

Declaration of Conflicting Interests

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Supplemental Material

The online data supplements are available at http://rs.sagepub.com/supplemental.

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