ERURJ, 2023, 2, 2, 320-330

Article

Dysregulation of Apoptotic Genes in Polycystic Ovarian Syndrome Patients Undergoing In-vitro Fertilization

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Received 18th March 2023, Revised 27th April 2023, Accepted 29th April 2023.

DOI: 10.21608/ERURJ.2023.200907.1016

ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a common endocrine-reproductive disease in females. Cell apoptosis plays an important role in follicular development, mainly in egg production, oocyte degeneration, follicle selection, or even follicle atresia. It also serves an important part in the embryo's early growth and differentiation into a complete organism. PCOS is a condition that is known to be caused by alterations in cell development and cell death factors, as well as oxidative stress, resulting in follicular interruptions and immature follicles. Apoptosis is a natural homeostatic process that helps the tissue remove the growing number of unwanted cells that are harmed during development, growth, or senescence. Caspases, an inhibitor of apoptosis proteins, the B cell lymphoma (Bcl)-2 gene family, the tumour necrosis factor (TNF) receptor superfamily, and the p53 gene are all participating and/or cooperating in the apoptosis pathway. Microarray data analysis of PCOS databases obtained from GEO datasets was performed to identify significant dysregulated apoptotic genes in PCOS oocytes compared to controls.

Keywords: Polycystic ovary syndrome (*PCOS), In vitro fertilization (IVF), Apoptosis.*

1-Introduction

Polycystic ovary syndrome (PCOS) is a prevalent hormone-related, condition that affects 6-21% of women (1). PCOS has an effect on women of productive age, and symptoms involve irregular or absent menstruation, hyperandrogenemia, and polycystic ovarian appearance, as well as disorders of metabolism such as insulin intolerance, diabetes, and obesity (2). PCOS is the leading cause of female infertility as a result of oocyte cell development failure (3).

Apoptosis, also known as programmed death, is the process by which any cell dies under certain or specified circumstances. Apoptosis is a normal balancing process that assists the tissue in eliminating the increasing quantity of unneeded cells that are damaged or no longer controllable during development, growth, or degeneration (4). This expected natural process is needed for the appropriate growth of organs during the formation of embryos as well as the elimination of abnormal cells, such as those harmed by exposure to pathogenic organisms or undergoing oncogenic change. The transition between cellular survival and apoptosis is closely controlled and essential to an organism's development as well as its health. Dysfunction in the apoptotic system, that inhibits cell death can result in embryonic anomalies or uncontrolled tissue growth (5).

Apoptosis is carried out by the external or the internal death triggering pathways, or in some instances by the perforin/granzyme B system, and leads to caspase cascade initiation. In the signaling mechanisms which encourage or suppress caspase activation, numerous classes of proteins are implicated (4). The current bioinformatics research mainly focused on the variety of gene families implicated in apoptosis processes in order to find the genes that are differentially expressed in PCOS oocytes versus controls.

2. Methods:

Microarray analysis:

The Gene Expression Omnibus (GEO) (6) is a database containing microarray, next-generation DNA sequencing, and other types of high-output genomic information that have been stored and are readily accessible for research purposes. GEO viz. GSE5850 provided the PCOS gene dataset. The dataset is based on Affymetrix GeneChip Human Genome U133 Plus 2.0 microarray chips (Affymetrix). Six normal women and six PCOS women having IVF gonadotropin treatment were involved in the study. Oocyte retrieval occurred 36 hours after hCG

injection. Each ovary's initial follicle was aspirated for follicular fluid. Each oocyte's total RNA was extracted and subjected to three cycles of linear amplification.

Statistical analysis

SPSS version 17.0 was used for the statistical assessment. (SPSS, Inc.). The Student's t-test was used to assess group differences. (2-sided). All quantitative findings are given as mean ± SEM. Using the Bonferroni correction, a two-tailed $P<0.05$ had been considered to suggest a statistically significant difference.

3- Results:

The microarray dataset revealed 189 apoptotic genes that were dysregulated in the oocytes of PCOS individuals. (Figure 1). Statistical analysis of the differentially expressed genes showed that 38 genes involved in the apoptosis regulation mechanism were significantly upregulated or downregulated (Table 1).

4. Discussion:

PCOS is the most common endocrinopathy disorder of women of productive age. Although patients with PCOS are typically characterized by an increased number of oocytes retrieved during in vitro fertilization, they are of impaired quality (7). PCOS individuals have fewer granular cells in their ovaries, which may be due to cell death to Bcl-2 downregulation and upregulation of Bax, Fas, and Fas-L (8). The goal of this research was to look into the apoptotic process in PCOS by using bioinformatics to investigate the gene expression of apoptotic and anti-apoptotic-related substances in PCOS oocytes.

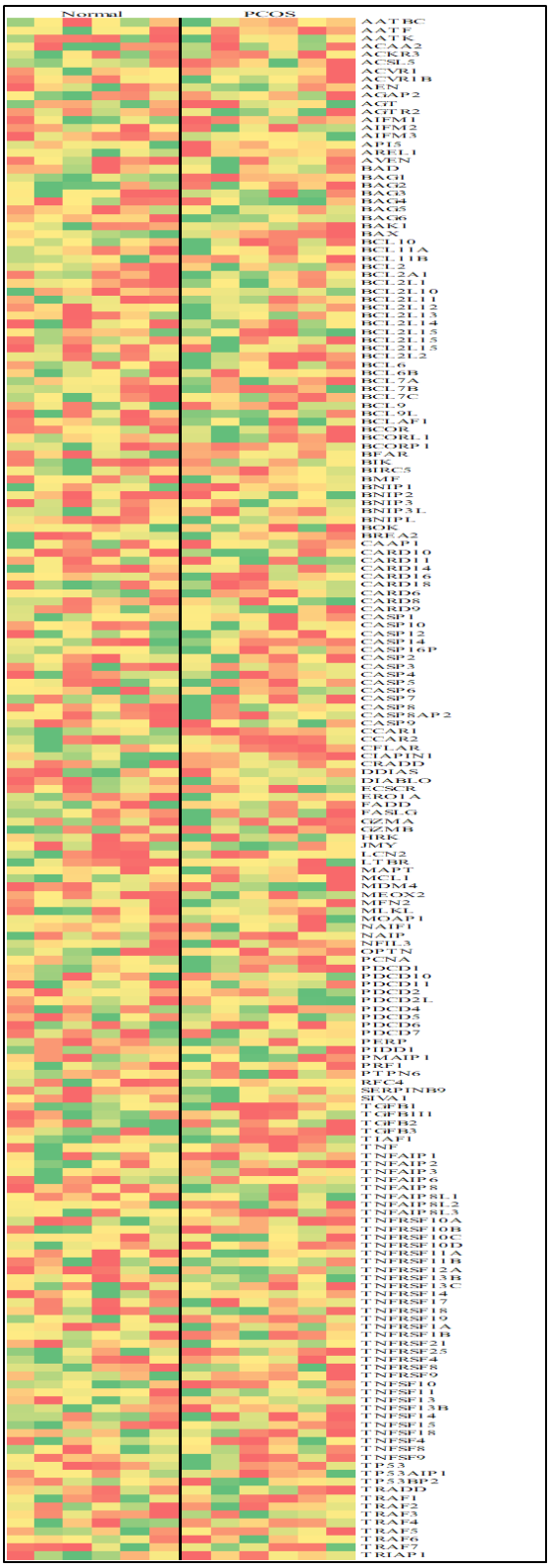

Figure 1. Heat map of the differently expressed apoptotic genes in PCOS oocytes samples and controls obtained from the GSE5850 dataset. Green represents upregulation; red represents downregulation**.**

Table 1. Significantly dysregulated genes involved in apoptosis mechanism and regulation with the observed fold change with q-value<0.05 in PCOS oocytes compared to control (GSE5850)

Gene symbol	Gene names	Fold change	q- value
AIFM1	Apoptosis-inducing factor, mitochondria associated 1	-2.16247	0.034136
AIFM3	Apoptosis-inducing factor, mitochondria associated 3	2.36747	0.033876
AREL1	Apoptosis-resistant E3 ubiquitin protein ligase 1	-2.093788	0.007647
BAG1	BCL2-associated athanogene 1	-3.039904	0.000183
BAG2	BCL2 associated athanogene 2	-1.633931	0.030312
BAG6	BCL2 associated athanogene 6	2.215291	0.001918
BAX	BCL2 associated X, apoptosis regulator	-5.384058	0.02881
BCL11B	B-cell CLL/lymphoma 11B	-2.683569	0.012674
BCL2L10	BCL2 like 10	1.498523	0.044007
BCLAF1	BCL2-associated transcription factor 1	3.324832	0.038827
BNIP1	BCL2 interacting protein 1	-2.837232	0.032039
BNIP2	BCL2 interacting protein 2	5.582884	0.04791
BNIPL	BCL2 interacting proteins like	3.260963	0.040125
CARD10	caspase recruitment domain family member 10	2.822312	0.017472
CASP1	caspase 1	-5.62844	0.049767
CASP8	caspase 8	7.594548	0.050148
CCAR1	cell division cycle and apoptosis regulator 1	-2.675799	0.000369
CCAR2	cell cycle and apoptosis regulator 2	-2.908117	0.039072
CFLAR	CASP8 and FADD-like apoptosis regulator	-2.107546	0.029752
CIAPIN1	Cytokine-induced apoptosis inhibitor 1	-2.054226	0.006113
MCL1	BCL2 family apoptosis regulator	-1.654489	0.032368
MDM4	MDM4, p53 regulator	4.922426	0.022788
MEOX2	mesenchyme homeobox 2	4.686825	0.021295
OPTN	Optineurin	-3.507212	0.051722
PDCD6	programmed cell death 6	-4.763265	0.037279
SERPINB9	serpin family B member 9	3.199068	0.039736
TNFAIP3	TNF alpha-induced protein 3	-1.532253	0.047798
TNFAIP8	TNF alpha-induced protein 8	5.48754	0.003823
TNFAIP8L1	TNF alpha-induced protein 8 like 1	2.528037	0.036363
TNFRSF10D	TNF receptor superfamily member 10d	-2.441715	0.033071
TNFRSF11A	TNF receptor superfamily member 11a	5.657884	0.037047
TNFRSF13B	TNF receptor superfamily member 13B	2.564472	0.024073
TNFRSF19	TNF receptor superfamily member 19	-3.183284	0.049873
TNFRSF9	TNF receptor superfamily member 9	-2.761404	0.034723
TNFSF10	tumor necrosis factor superfamily member 10	-2.452758	0.03464
TNFSF11	tumor necrosis factor superfamily member 11	3.813036	0.046553
TRAF3	TNF receptor-associated factor 3	3.117418	0.019321

Apoptosis is thought to be an essential component of many processes, including regular cell renewal, immune system growth, and function, hormone-dependent degeneration, growth and development of embryos, and chemical-induced cellular death. Apoptosis which is either too little or too much is an underlying factor in many chronic diseases (4). Apoptosis is carried out by the external or internal death-triggering pathways and concludes in caspase cascade activation. In the signaling pathways that aid or prevent caspase activation, multiple protein families are implicated. (Figure. 2).

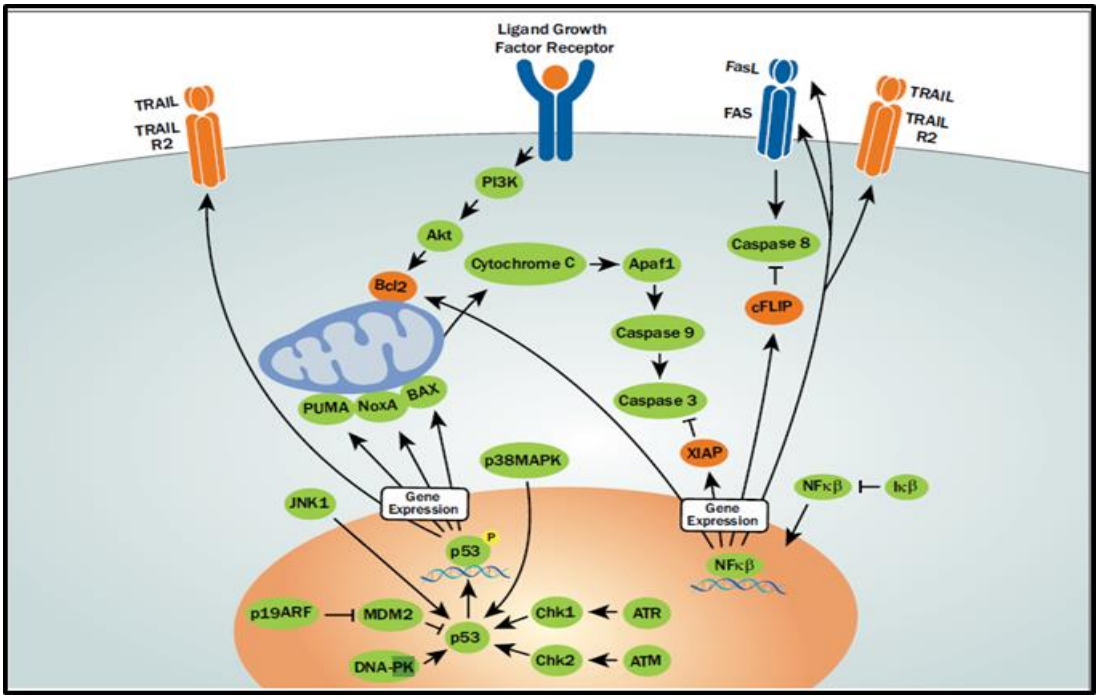

Figure 2. The most important apoptotic routes; internal and external approaches (5)

The association between cell death receptors and their chemical ligands starts the extrinsic path, which results in caspase 8 activation. The intrinsic path, on the other side, can be activated by DNA degradation. As a consequence, the cells are able to trigger apoptosis via the mitochondrial pathway (9).

Upon analyzing the data set (GSE5850), dysregulation in the expression of the following important genes involved in the apoptosis mechanism of PCOS oocytes compared to control was observed as AIFM3, BAG6, BCL2L10, BCLAF1, CASP8, MDM4, MEOX2, TNFAIP8, TNFAIP8L1, TNFRSF11A, TNFRSF13B, and TNFSF11 that significantly upregulated while

AREL1, BAG1, BAG2, BCL11B, CIAPIN1, MCL1, and OPTN that significantly downregulated. In PCOS the loss of embryo cells due to inappropriate or excessive apoptosis may be deleterious.

AIFM3 is a mitochondrial protein with a molecular weight of 66 kDa and 598 amino acids. AIFM3 shares 35% of its sequence with the apoptosis-inducing factor (AIF). AIFM3 is also capable of inducing apoptosis (10).

BAG6 (also known as BAT3/Scythe) is a ubiquitin-like protein suspected to be involved in a number of unrelated physiological and pathological processes, particularly apoptosis (11).

In addition to its anti-apoptotic action, BCL2L10 may play additional functions in cell cycle regulation and oocyte maturation. Maternally inherited proteins regulate several embryonic processes. BCL2L10, the most abundant maternal protein in the BCL2 family in oocytes, decreases over time after fertilization, indicating that BCL2L10 may play important roles before and during fertilization, during the oocyte-to-embryo transition, and the very early embryonic stages (12).

BCLAF1 has been linked to apoptosis by either promoting the transcription of its downstream target genes [such as TP53 and Bax] or inhibiting the transcription of its downstream target genes (such as MDM2) (13).

Caspase-8 (CASP8) is known to spread the apoptotic signal either directly by cleaving and activating downstream caspases or indirectly by cleaving the BH3 Bcl2-interacting protein, which results in the release of cytochrome c from mitochondria, activating caspase-9 in a complex with dATP and Apaf-1 (14).

MEOX2 overexpression reduces cell viability and promotes apoptosis through modulating apoptosis and PI3K/Akt pathway-related proteins (15).

Death receptors (DRs), which are classed in the TNF superfamily and include ligands, are one of the most significant means of inducing apoptosis (16). The TNF superfamily is known to have 19 ligands and 29 receptors that act in a variety of physiological processes like inflammation, apoptosis, proliferation, and invasion (17) so genes encoding TNF superfamily receptors and ligands significantly contribute to apoptosis.

Downregulation of anti-apoptotic genes in PCOS embryos increases their susceptibility to death and deteriorates their viability.

The Bcl-2-associated athanogene (BAG) family, which includes BAG1 and BAG2, is a collection of proteins that inhibit cell death by interacting with Bcl-2 (18). AREL1 encodes an

E3 ubiquitin ligase that restricts the cellular response to apoptotic stimuli by ubiquitinating proapoptotic proteins after mitochondrial release and directs their destruction via the proteasome (19).

Small interfering RNA (siRNA) downregulation of the BCL11B gene resulted in growth inhibition and apoptosis (20). Mechanisms of BCL11B-loss-induced apoptosis may be linked to the mitochondrial apoptotic pathway, and identified cleavage and fragmentation of recognized caspase targets, including myosin, spectrin, vimentin, and ERM proteins, which were up-regulated and phosphorylated following BCL11B depletion, may be implicated (21).

Cytokine-induced apoptosis inhibitor 1 (CIAPIN1) is a newly discovered anti-apoptotic molecule that is found in both the nucleus and the cytoplasm of most tissues. CIAPIN1 prevents cytokine-deprivation-induced apoptosis. When cytokines interact with receptors, they activate receptor tyrosine kinases and send mitogenic and anti-apoptotic signals via downstream signaling pathways (22).

Mcl-1, an anti-apoptotic Bcl-2 protein, promotes neural precursor cell (NPC) survival in both the developing and adult mammalian neurological systems. At embryonic day 9.5 (23), the loss of Mcl-1 caused an influx of apoptosis to start in the brainstem and cervical spinal cord. Also, optineurin (OPTN) controls ER stress-induced signaling pathways and protects against ER stress-induced cell death. (24).

5. Conclusion

PCOS oocytes may have a higher apoptosis index compared to control which can affect the embryo quality, viability, and pregnancy outcome. This study concluded that some apoptotic and anti-apoptotic genes can be considered markers for the viability of embryos before implanting them in the uterus or using them as a therapeutic target to increase the vitality of embryos taken from PCOS patients.

- **Conflict of Interest**

The authors declare no conflict of interest.

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