Adjustment of expression of TUBB8, SOX9 and BCL2 genes in apoptosis and polycystic ovarian cancer

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ABSTRACT
Polycystic Ovary Syndrome (PCOS) is the most common heterogeneous Endocrine (EC) disorder in women of childbearing age, which has an increasing trend in the number of patients recently. Although women with PCOS have been shown to triple the risk of EC compared to women without polycystic ovary syndrome, there are precise molecular mechanisms that increase the risk of EC in women with ovarian syndrome. Therefore, clinical strategies to prevent EC in PCOS are not well known. Although, elevated estrogen levels and decreased apoptosis have been suggested as potential mechanisms, there is no clarity on how these and other factors interact to increase the risk of EC in polycystic ovary syndrome. In this article, we try to review the functional mechanisms of TUBB8, SOX9 and BCL2 genes to examine their effect on controlling or promoting the risk of EC.

Keywords: TUBB8, SOX9, BCL2, polycystic

INTRODUCTION
Polycystic ovary syndrome (PCOS) is the utmost commonplace heterogeneous endocrine disease in ladies, especially those of childbearing age. PCOS causes hormonal imbalances that, if continued, can lead to irregular menstrual cycles,
numerous cysts, and eventually infertility. PCOS affects 5 to 15% of women worldwide and may be a risk factor for cancer development, as previous observations, have shown conflicting results in PCOS and ovarian cancer risk. Most of these studies have shown a useless or suggested increase in the risk of ovarian cancer. It is noteworthy that many candidate genes have been identified as a factor in PCOS in previous studies [1,2].

Tubulin genes family is one of these genes which is thought have some affect in process of PCOS. In a study that looked at changes in the tubulin genes in tumor samples, it was found that the TUBD1 and TUBB3 genes of this family had the highest proliferation and deletion in breast cancer tumors, respectively. TUBB8 is a special beta-tubulin isotype found only in mammals. Recent research has found that this gene is the predominant isotype in the human oocyte, and mutations in it can stop the maturation of the human oocyte [3].

Another gene family include BCL2, which encodes mitochondrial proteins and prevents normal cell apoptosis. In various types of cancer (e.g. in 0.5< of breast cancers) expression of BCL2 gene has been observed. BCL2 genes and their product have an impressive role in stimulating growth, survival and inhibition of apoptosis. Some studies have shown that BCL2 decreases in invasive breast cancer compared to normal breast and pre-invasive breast lesions, and BCL2, as an apoptotic inhibitor, should be associated with highly invasive tumors resistant to hormone therapy. Expression of BCL 2 excessively in response to cell intoxication, is also related with cancer and decreased apoptosis [4].

In the early 1990s, a new transcription factor was discovered that played a key role in determining the testis. The gene encoding it was called SRY. SOX9 ( E subset of the SRY protein) is an essential transcription factor. In recent years, there has been growing evidence that SOX9 can regulate many growth pathways by the expression of numerous genes, (including proliferation, apoptosis, migration, invasion, angiogenesis, metastasis, etc). Extensive studies have also shown SOX9 to be involved in various cancers such as brain cancer, bladder cancer, esophageal cancer, endometrial cancer, stomach cancer, ovarian cancer, prostate cancer and more [5].

The role of these genes in the development of various cancers mentioned above, led us to examine the functional mechanisms of
Ahmadifar et al.
the TUBB8, SOX9 and BCL2 genes and their role in controlling or increasing the risk of ECOS in people with polycystic ovary syndrome.

The cellular and molecular basis of cancer in humans
If any mechanisms involved of the in controlling the normal growth of cells are disrupted, cancer can occur. The lack of control over cell division mechanisms that are responsible for most or all of the cancers is due to mutations and genetic damage [6]. These damages are often caused by radioactive chemicals, hormones, and sometimes viruses. Evidence that two distinct types of viruses can cause cancer was first proposed in 1911 by Python Ross. There are viruses with DNA genomes, like papilloma and adenoviruses, and those with RNA genomes that termed retroviruses. Human T-cell leukemia viruses (HTLVs) and the related retrovirus, human immunodeficiency virus (HIV) are the only currently known human retroviruses. Retroviruses can induce a transformed state in host cells by two mechanisms related to their life cycle. Transformation of viral RNA genomes into DNA occurs after host cell infection with retrovirus. The produced DNA then integrates with the host genome and proliferates with the

Expression of TUBB8, SOX9 and BCL2 genes
host cell divisions. Powerful transcriptional promoter sequences, called long-term repeats (LTRs), are located within the end-sequence sequences of the retroviral genome, enhancing the production of new viral particles [8].

It is noteworthy here that the second mechanism used by retroviruses is related to LTRs, which result in the localization of LTRs close to the gene that encodes the growth-regulating protein. If protein expression is abnormally elevated it can lead to cellular transformation. This is called retroviral integration, and it has recently been shown that certain forms of cancer in people infected with this process are caused by HIV [9].

Genes such as BRCA-1, TP53 and BRCA-2 are involved in normal cell division and DNA repair. They are very important to detect inappropriate growth signals or DNA damage to cells. If these genes are unable to function as a result of inherited or acquired mutations, the DNA integration monitoring system becomes dysfunctional, causing the mutated cells to spontaneously stabilize and proliferate, forming tumors [10]. Normal cells contain proteins such as cyclin-dependent kinases (CDKs) to maintain and control cell cycle proliferation and progression. CDKs
Ahmadifar et al. activate other downstream kinases by phosphorylating which leads to drive progression of different cell phases. CDK activity is possible only in the presence of cyclins (activating subunits) synthesized following a cell cycle-dependent manner. The predominant regulators of Cyclin-CDK complexes are CDK inhibitors [11].

In normal cells, growth factors (GFs) and their receptors (GFRs) are involved in controlling normal growth and maintaining tissue homeostasis. But in cancer, their function often goes derail. As a result of their derailment function, they allow wayward cells to produce their own internal signals. This process stimulates proliferation and causes wayward cells to become independent of their environment. Cancer cells can induce their growth-promoting signals when a mutation in the GFR gene occurs, which facilitates activation in the absence of GFs, or when overproduction of GFs leads to an autocrine signaling loop [12].

Mutations in three broad genes cause the onset of cancer:

1) **Proto-oncogenes:** Proto-oncogenes are generally responsible for regulating cell division and growth and naturally accelerate cell growth. When...

**Expression of TUBB8, SOX9 and BCL2 genes** mutations occur in these genes, they become oncogenes that have very high gene expression, and in various ways, including direct and continuous stimulation of cell surface growth factor receptors, intracellular signal transduction pathways, transcription factors, etc cause the growth process to accelerate more than normal. Factors that convert proto-oncogenes to oncogenes: 1) Chromosomal Translocation 2) Point mutation 3) Deletion 4) Amplification 5) Insertional activation.

All of these proto-oncogene-to-oncogene-converting factors may increase or alter the activity of a gene product [13]. Many oncogenes have been identified that may be involved in human neoplastic malignancies. For example, the Ras protein (encoded by the RAS gene), the RAS-MAPKinase pathway, carries signals from membrane-bound receptors to the cell nucleus that regulate cell division. Mutations may result in the in proper activation of the Ras protein, cause uncontrolled cell growth. Ras
Ahmadifar et al.

protein has been shown to be abnormal in some human cancers (about 25%) [14]. Other oncogenes involved in certain cancers include HER2 (boosted in stomach, breast, and to a lesser extent in lung cancer); BCRABL1 (a translocation of 2 genes that underlies chronic myeloid leukemia and some B-cell acute lymphocytic leukemias); CMYC (Burkitt lymphoma); NMYC (small cell lung cancer, neuroblastoma); EGFR (adenocarcinoma of the lung); EML4ALK (a translocation that activates the ALK tyrosine kinase and causes a unique form of adenocarcinoma of the lung) [15,16, 17, 18].

2) Repair genes: Repair genes naturally make proteins and enzymes that have the ability to repair damaged genes. Once mutated, they can no longer repair the defects of other genes. All cell genes are naturally attacked by environmental and metabolic factors, and as a result of this successive damage, the genes are in dire need of repair proteins. To date, more than 30 types of repair proteins with significant roles have been identified to correct genetic defects in cells. More than one million genetic damage is done to the genes of each cell every day, and if these defects are not repaired, the cell will either age, become apoptotic, or develop cancer. The best example is the BRCA-1 repair gene. This gene makes a protein with several properties, including the ability to modify defective genes. This protein contains the Zinc finger molecule that controls the expression of dependent genes [19]. BRCA-1 and RDA-1 proteins can repair DNA strand breaks. The BRCA-2 gene, also makes a protein that acts like the BRCA-1 protein. An increased risk of breast and ovarian cancer can be seen following mutations in these genes and reduced function [20].

3) Supressor genes: Lack of tumor inhibitory genes causes uncontrolled division of cancer cells. The P53 inhibitor protein is located on the TP53 gene on chromosome 17 P 13/1. The length of this gene is 20,000 bps, which makes a protein 393 amino acids
Ahmadifar et al.

long. An important regulatory protein, P53, prevents damaged DNA from replicating in normal cells and promotes cells with abnormal DNA to progress to apoptosis. Mutated p53 (inactivated or altered) allows cells with abnormal DNA to divide and survive, according to which the P53 gene mutation is found in more than 60% of cancerous tissues. Mutations in the TP53 gene are also inherited in daughter cells. More than 35 types of inhibitory genes have been identified and reported so far. P53 protein, which acts as both an inhibitor and a stimulator of cancer cells, plays an important role in the production of cancer cells [21]. This protein creates a network of molecular events. After damage to other genes, the P53 protein binds to DNA to stimulate the expression of the WAF1 gene, which makes the P21 protein and binds to the CDK2 protein. P53 prevents P21 from entering the next stage of cell division. The active P53 protein is phosphorylated by N-terminals in two ways [22,23]. Through protein MAPK and through ATM, ATR and LHK protein. When P53 is phosphorylated, it loses its adhesion to MDM2. The protein deforms the structure of P53 and helps to prevent P53 from binding to MDM2. When the P53 gene is free of environmental shocks, the value of P53 goes down. The MDM2 protein binds to P53, inhibits its action, and transfers it to the cell cytoplasm [24, 25].

The anti-cancer action of P53 can be done in three ways:

1. P53 stimulates DNA repair proteins to compensate for gene damage.
2. P53 protein stimulates programmed death (when damaged cells are non-regenerating).
3. P53 protein maintains cell division in the G1 / S stage as an opportunity for repair.

Another suppressor gene is the retinoblastoma (RB) gene which encodes the RB protein that has a regulatory role in the cell cycle by stopping DNA replication. Mutations in the RB gene family have been observed in many human cancers, which allow damaged cells to divide continuously [26].
Genetic changes that occur in cancer cells affect almost all aspects of homeostasis, cell proliferation, tissue organization, migration, survival, and proliferation in external areas of the body. Cancer cells often show the characteristics of fast-growing cells, including: High ratio of nucleus to cytoplasm; Prominent cores; Increased cells in mitosis; A structure that is relatively less specialized. Tumor cells have significant differences in appearance, composition of all proteins, and therefore cellular function compared to normal cells. Other common features of cancer cells include uncontrolled proliferation. Most cancer cells rely on glycolysis for energy, regardless of whether the oxygen level is high or low [23]. In cancer cells, growth-promoting pathways are up-regulated, while at the same time, growth inhibitory pathways and apoptosis are down-regulated. In this way, cancer cells become capable of continuous proliferation. Normal cells stop growing after contact with other cells. As a result, a regular cell layer is formed, but the cancer cells have less adhesion and form 3D clusters of cells. In some cancers, normal tissue stem cells may become cancerous stem cells. In some other cancers, the dedifferentiation of completely differentiated cells may lead to the formation of progenitor cells and eventually cancer stem cells. Cancer cells, regardless of origin, are sharing the gene expression patterns with normal tissue stem cells, thereby recent cells becoming stem cell-like cells [13].

Telomeres are nucleoprotein complexes that cover the physical ends of linear chromosomes and maintain their integrity by forming the successive arrangement of a short DNA sequence (TTAGGG in vertebrates). In normal tissue, telomere shortening due to aging causes a slight restriction in cell division. The telomerase enzyme is a type of reverse transcriptase that contains an RNA template that repeatedly adds TTAGGG repeats to the ends of chromosomes and maintains or prolongs the repeats at the ends of human chromosomes [28]. If the telomerase enzyme is activated in tumor cells, it allows new synthesis of telomerase and the continuous proliferation of cancers. Embryonic cells, germ cells, and stem cells produce telomerase, while most human somatic cells produce only a small amount of telomerase as they enter the S phase of the cell cycle. Due to the low telomerase activity in these cells, the telomere length is gradually reduced during each period of cell division to the point that this shortening causes the double-stranded DNA to be broken.
Ahmadifar et al.

Expression of TUBB8, SOX9 and BCL2 genes during waterfall invasion. First a local invasion occurs, then the cell invades the blood / lymphatic vessel wall (intravasation) and is transmitted to distant tissues through the blood / lymph vessels. Cancer cells then escape from the bloodstream (extravasation) and gain the ability to adapt to the local tissue environment and proliferate in that tissue [31]. A secondary or metastatic tumor results from the metastasis of tumor cells, called a new tumor, and its cells resemble primary or primary tumor cells. When cancer has metastasized, treatment options are greatly reduced, or often eliminated altogether [32].

Clinical and pathological observations

Among all female reproductive system cancers, ovarian malignancies have undergone extensive clinical trials. Several scientific developments have recognized that ovarian cancer is not a homogeneous disease, but a group of diseases that appear with different morphology and biological behavior. Ovarian epithelial cancers are the most common ovarian malignancies because they usually remain asymptomatic until metastasis, and in more than two-thirds of patients at the time of diagnosis, the disease is advanced [33]. This cancer has the highest mortality rate among all female reproductive system cancers and stopping the cell cycle and apoptosis. Tumor cells overcome this fate by producing telomerase [29].

Tumors feed and grow by inducing the formation of blood vessels called angiogenesis. Tumors in terms of size and the rate of localization, divided into two categories: 1. Benign 2. Malignant. Some types of benign tumors that affect skin cells include warts. Benign tumors are very similar to normal cells in the body and can only become a serious problem if their large volume disrupts normal functions or excessive amounts of physiologically active substances (such as hormones) to secrete. The distinguishing feature of benign and malignant tumors is the ability of malignant tumors to invade adjacent cells and tissues and to proliferate and spread in parallel with cell division. Some malignant tumors, such as breast and ovarian tumors, remain localized and encapsulate for at least a period of time, but as they progress, they can invade and metastasize to surrounding cells and tissues [30].

If cancer cells spread through the bloodstream / lymph to distant parts of the body, they are said to have metastasized, a process that is the deadliest form of cancer. Invasive-metastatic stages occur
changes in the methylation pattern in the promoter of genes are other cases of ovarian cancer. Hyper methylation, which occurs in the promoter of tumor suppressor genes, causes the gene to be silenced and can be used as a marker for early detection. This event can be used as a marker for early detection. The Infection risk of ovarian cancer all over the women's lives is about 1.5 percent and the risk of death from ovarian cancer is about 1 percent. Anatomically, the ovary has three major parts: the outer cortex, the central medulla, and the ovarian network [34].

The ovarian umbilicus is the point where the ovary joins the mesovarium. In the steroidogenesis, parts including nerves, blood vessels and umbilical cord cells can be active. The outermost part of the cortex, called the tonica albuginia, is covered by a layer of cubic epithelium [35]. This layer is called the superficial ovarian epithelium or ovarian mesothelium. Approximately 90% of ovarian cancers are ovarian epithelial cancers that are heterogeneous and their classification is based on the type of cell involved, which includes serous, mucinous, endometrial, clear cell, brenner, and various types of epithelial cells. Each of these tumors is in turn divided into three groups: benign, interstitial and malignant. Mucosal and endometrial tumors are usually malignant and invasive carcinomas. But serous tumors are not usually invasive [36].

Molecular genetic studies to determine a pattern for ovarian cancer have shown that there are two pathways for tumorigenesis (pattern 1 and pattern 2).

- Pattern 1: Contains low-progression tumors of serous carcinoma, mucinous carcinoma, endometrial carcinoma, tumor malignant brenner, and clear cell carcinoma that their growth is gradual. They usually originate from a primary precursor, grow slowly, and are confined to the ovaries (they do not have metastasize).

- Pattern 2: They are diagnosed when they are in advanced stages and include advanced serous carcinoma, mesodermal tumor. They usually do not originate from the primary precursor, evolve rapidly, they are metastasizing and invader. Like in Model 1, their growth is gradual and only have a higher growth speed. The reason for the high
Expression of TUBB8, SOX9 and BCL2 genes reporting of cancer pathology. All the elements needed for a standard pathological cancer pattern are significant, even if some of them seem to be "minute" or less important. These desirable characteristics characterize ovarian cancer that have been reported in various studies include the following: lymphovascular attack of cell carcinomatosis, type of ovarian capsule rupture or carcinomatous invasion, and peritoneal tumor spread (with adequate biopsy of non-invasive or invasive implants in borderline or carcinoma-type tumors) [40].

Epigenetic changes are biomarkers for early detection, control of disease progression, and markers for response to treatment. Examination of epigenetic changes, including changes in the methylation pattern, can play an important role in the pathology of ovarian cancer. The benefits of this study include the following [41]:

1. Changes in DNA methylation is a bilateral sign that indicates a detectable cancer cell at low density.
2. Epigenetic changes occur in only one part of a gene, so there is no need to thoroughly examine a gene for detect the mutations.
Ahmadifar et al.

3. Since DNA is used to analyze methylation events, the results can be cited because DNA is much more stable than RNA and protein.

Clinical and pathological aspects of PCOS

Oligomenorrhea, hirsutism, hyperandrogenism, and obesity with polycystic ovary enlargement (PCOS) are criteria for diagnosing PCOS. These problems are caused by persistent ovulation with a range of clinical causes and manifestations, and its clinical aspects are now recognized as a heterogeneous disorder that results in the overproduction of androgens, primarily from the ovaries, and is associated with insulin resistance. PCOS may be correlated with amenorrhea, infertility, hyperandrogenemia (HA) and signs of metabolic disorders such as insulin resistance and dyslipidemia. Adolescents usually have relative androgenemia, insulin resistance, cystic ovaries, and ovulatory cycles that later transits to estrogen state during puberty. Failure to make this transition may lead to PCOS secondary and abnormal development during puberty [42]. Ovulation is a pathognomonic feature of PCOS and leads to irregular menstrual cycles. Menstrual irregularities lead to oligomenorrhea. Less than 9 menstrual cycles per year as well as cycles with an average length of more than 36-40 days have been observed in these patients. An imbalance in various hormonal functions can affect ovarian homeostasis and leads to ovulation, which will manifest as PCOS [43].

One of the features of this syndrome is biochemical and clinical hyperandrogenism in the ovary, which is observed in 60 to 80% of patients. Ovarian hyperandrogenism is mostly due to a defect in the intrinsic steroid synthesis in ovarian theca cells. Extra-ovarian factors such as high levels of LH and insulin and low levels of FSH, as well as intra-ovarian factors such as anti-Müllerian hormone (AMH) and inhibin, may contribute to the reinforcement of hyperandrogenism. Hyperandrogenism with an instantaneous impact on insulin signaling can be one of the viable causes of insulin resistance in sufferers with polycystic ovary syndrome. High levels of androgens during intrauterine life and immediately after birth may lead to accentuate visceral adiposity as well as insulin resistance. Insulin resistance and compensatory hyperinsulinaemia are involved in all three major clinical aspects of the syndrome: hyperandrogenism, ovarian dysfunction,
Ahmadifar et al.

and metabolic changes. An increase in LH pulse leads to increased circulating LH levels, which stimulate the synthesis of androgens in the ovarian cortex. The increase in LH levels are partly due to the negative feedback exerted by androgens on the hypothalamic-pituitary axis [45].

Insulin, in conjunction with LH, increase the stimulation of androgen production by the ovary theca cells and to a lesser extent by the adrenal cortex, and may play a role in ovarian dysfunction by increasing the expression of LH receptors in granulosa cells. The first treatment for obese patients with PCOS is weight loss because obesity is common in these people. In addition to improving obesity-related metabolic comorbidities, weight loss reduces hyperinsulinaemia, resulting in increased insulin sensitivity, decreased LH and androgen levels, and improved menstrual and fertility cycles. The danger of metabolic disorders will increase in obesity and additionally has a bad effect on insulin resistance [46].

One of the clinical manifestations of hyperandrogenism, hirsutism, is male pattern hair growth in women with PCOS. Hirsutism is a common disorder caused by androgenic activity, which in women is characterized by excessive growth of terminal hair in androgen-dependent regions of the body. Androgens are partly responsible for promoting the anagen phase (growth phase) in the hair cycle, which causes hair follicles to enlarge. The anagen phase is affected by insulin-like growth factor (IGF-I) [47]. Thus, women with PCOS may exhibit disorders in the metabolism of the two main factors responsible for hirsutism, which are androgens and insulin growth factors. Measuring testosterone levels can show the best results for reflecting androgen status. Other laboratory activities performed to check androgen levels include [48]:

- Free Androgen Index (FAI), which is the ratio between total testosterone and Sex hormone-binding globulin (SHBG).
- Androstenedione is a direct precursor to testosterone produced by the ovaries, adrenal glands, and peripheral tissues. In women with PCOS, androstenedione levels can rise even if total testosterone is normal.
- Adrenal DHEA-S levels increase by about 20-30% in patients with PCOS. Hyperandrogenism, by increasing the activity of the

Expression of TUBB8, SOX9 and BCL2 genes

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Ahmadifar et al.

Expression of TUBB8, SOX9 and BCL2 genes

Ovarian cancer is one of the main causes of cancer mortality in women worldwide. Despite the higher resistance of tumors to therapeutic drugs, standard treatment for this cancer includes platinum-based chemotherapy. One of these mechanisms of drug resistance is to alter the molecules involved in apoptosis and help cells escape death. We first briefly examine apoptosis and its pathways. Apoptosis or programmed cell death has two main pathways, which include the internal or mitochondrial pathway and the external or death receptor pathway. The intrinsic pathway is activated by intracellular signals when cell pressures occur, and the stress signals received by intercellular molecules mediate the permeability of the mitochondrial outer membrane (MOMP) and release pro-apoptotic molecules into the cytoplasm. The two primary components of the innate apoptosis pathway are the B-2 cell lymphoma family (BCL-2) and apoptotic protein inhibitors (IAPs), which control mitochondrial membrane permeability. The external pathway originated by binding extracellular ligands to cell surface death-receptors, leading to the formation of the death-inducing signaling complex (DISC). The external pathway begins through the association of cell surface receptors, a

P450C17 enzyme complex in theca cells, 17α-hydroxylase, which converts progesterone to 17-OH-P, and 17-20 lyase, which transforms the latter to androstenedione. Slowly Androstenedione is then converted to testosterone by β-hydroxysteroid dehydrogenase17. Insulin is able to directly stimulate the enzymatic activity of P450C17, both at the adrenal and ovarian levels. Total androstenedione and testosterone levels help to better assess the risk of metabolic syndrome in women with PCOS [49].

Inositol metabolism is altered in women with PCOS. The conversion of myo-inositol to d-chiro-inositol is reduced at the level of muscle tissue in these patients due to decreased epimerasic activity. In these patients, serum D-chiro-inositol levels also decrease and urinary excretion inositol phosphoglycans increases.

In general, the clinical aspects of this disease represent a chain of pathological and hormonal reactions. Timely therapeutic intervention can stop this ongoing process.

Apoptosis in ovarian cancer (OvCa)
subset of tumor necrosis factor (TNF) with the corresponding activator cytokine ligands of the TNF-superfamily of proteins. Apoptosis may be very essential for ovarian function because it regulates periodic processes in the female reproductive system [50]. For example, apoptosis has been observed in germ cell attrition before ovulation, follicular atresia, Corpus luteum regression (Luteolysis) and ovarian surface epithelial cells. Seven million oocytes formed in the ovaries early in human embryonic life are reduced by about one-third by apoptosis immediately after birth. Defects in apoptosis or persistent proliferation of germ cells may lead to germ cell tumors in the ovary. The primary motive of cell death in follicular atresia is granulosa cell apoptosis. An excessive increase in follicle-stimulating hormone (FSH) prevents the death of apoptosis in some antral follicles during follicle growth. Few ligands and receptors are involved in the dominant follicle ovulation process. They are: Tumor Necrosis Factor alpha (TNF-α), TNF-related apoptosis inducing ligand (TRAIL), Fas ligand and APO-3 ligand which induce apoptosis via the external pathway [51]. Tumor initiation, transformation, or metastases has been shown to be caused by some oncogenic mutations that disrupt apoptotic mechanisms. In addition, most cytotoxic chemotherapy causes apoptosis, which indicates that the treatment failure process is significantly due to dysfunctional apoptotic mechanisms. Targeted therapy is such that it can control apoptotic pathways by targeting specific genes and proteins that regulate these pathways. Therefore, targeting apoptosis remains the main focus and safest way towards combating fight cancer [52].

We now turn to the role of the Bcl-2 family in apoptosis and ovarian most cancers, which might be vital regulators of the innate pathway of apoptosis. They classify into three subgroups depending on their function: proteins that can induce apoptosis, known as pro-apoptotic, those that inhibit apoptosis, called anti-apoptotic and pro-apoptotic Bcl-2-homology (contains only BH3 proteins). Pro-apoptotic proteins, which are B-cell associated X protein (Bax), Bcl-2 homologous antagonist killer (Bak) and Bcl-2 related ovarian killer (BOK), contain a range of 1 to 4 BH. Bcl-2 binds to Bax, prevents c-Myc-induced apoptosis, and also blocking the release of cytochrome-c in mitochondria. This compound inhibits the interaction of the Apoptotic protease-activating factor
Ahmadifar et al. 1(Apaf-1) with caspase-9. In the ovaries, Bcl-2 is expressed primarily in healthy ovarian follicles, while Bax, a pro-apoptotic molecule, is expressed in atretic follicles. High levels of gonadotropins inhibit Bax expression while increasing Bcl-2 and Bcl-xL expression, thereby enhancing follicle survival. Other pro-apoptotic molecules, such as Mcl-1, Bax, and Bok, exert their apoptotic effects by stimulating mitochondrial cytochrome-c secretion. Cytochrome-c binds to Apaf-1, forming the apoptosome and subsequently activates the caspase cascade which leads to apoptotic cell destruction. Bax and Bok initiate apoptosis by forming an oligomer on the outer mitochondrial membrane, leading to the MOMP [53,54]. Ovarian cancer is associated with mutations in some genes caused by apoptosis, and restoring their function can be alternative treatment options, hence small molecules as potential treatment options for targeting Bcl-2 and as a result, we use it to inhibit its activity in cancer cells. One of the molecules that act as a selective inhibitor of Bcl-2 is AB-737, whose oral bioavailability analogue (AB-263) inhibits cell growth in eight different ovarian cancer cell lines. Further experiments have shown that combination therapy of AB-737 with carboplatin increases the sensitivity of several cell lines to carboplatin. Another molecule that targets Bcl-2 is AT101. The importance of this molecule, unlike AB-737, is that it binds with high affinity to Bcl-2, Bcl-xL, and Mcl and it induces cell apoptosis by activating Bax through a modifying conformational change, translocation and oligomerization, leading to cytochrome c release and caspase-3 cleavage [55].

Other apoptotic regulatory proteins include apoptotic protein inhibitors (IAPs), which are also involved in immunity, inflammatory cell cycle regulation, and migration. The IAP inhibits the caspases by binding the Baculovirus IAP Repeat (BIR) domain to active caspase sites, a class of cysteine proteases involved in propagating apoptotic signals within the cell. X-linked apoptosis inhibitor protein (XIAP), the most potent inhibitor of apoptosis among all IAPs, is a regulator of apoptosis. By binding and inhibiting upstream caspase-9 and the downstream caspase -3 and -7, it inhibits both intrinsic and also extrinsic apoptotic pathways. In normal ovaries, surging FSH upregulates XIAP which in turn suppresses apoptosis of ovarian granulosa cells and increases the growth of FSH-induced follicles. Inhibition of XIAP, because it's far involved inside the chemical resistance of
Ahmadifar et al. ovarian cancer, is a mechanism that leads to extended apoptosis in platinum-resistant ovarian cancer cells in vitro and in vivo [56]. The mechanism of platinum-induced apoptosis involves the secretion of cytochrome-c, second mitochondria-derived activator of caspases (SMAC) and TNF-α. One way to induce apoptosis is to use SMACs, which are mitochondrial proteins that bind to IAP. They are released in conjunction with cytochrome c all through the innate apoptotic pathway inside the cytosol. This protein induces apoptosis by neutralizing the inhibitory effect of IAP on the processing and activity of caspases. There are eight IAP members in mammals; However, most investigated IAPs are cIAP1 and cIAP2 which bind to tumor necrosis factor receptor-associated factor 2 (TRAF2) to block the caspase-8 activation complex (apoptosis process depends on caspase-8) and inhibit TNF-induced apoptosis. Caspase 8 activates effective downstream procaspases, including procaspase -3, -6, and -7 leading the activation of specific kinases [57]. TNF-α, the main ligand of the TNF superfamily, plays a vital role in inflammation, apoptosis, and immune system development. In normal ovarian development, the expression of some members of the TNF family, such as FasL / Fas, is greatly influenced by gonadotropin levels. Surging gonadotrophin levels reduces Fas / FasL expression and results in follicular survival. However, decreased gonadotropin levels lead to increased Fas / FasL expression as well as follicular atresia. TNF-α, which is highly expressed in ovarian cancer, induces apoptosis by activating caspases and also regulates CD44 expression in T lymphocytes, which are involved in carcinogenesis and metastasis of ovarian cancer. On the other hand, TNF-α is able to activate the Nuclear factor-κB (NF-κB) survival pathway or caspase-dependent cell death by binding to Tumor necrosis factor receptor type 1(TNFRI). Activated TNFRI recruit TNFR-associated death domain (TRADD) which in turn recruits TNFR-associated factors (TRAFs) and Receptor-interacting protein (RIP) to activate NF-κB. Active NF-κB promotes the expression of genes that keep cell proliferation active, while protecting the cell against apoptosis [58]. TNFRI also forms a cytoplasmic TRADD complex containing FAS-associated death domain (FADD) that activates caspase-8 by stimulating procaspase-8, leading initiation of an apoptotic signaling cascade. Tumor necrosis factor-related
Apoptosis-inducing ligand (TRAIL), which is expressed in growing, atrial and antral ovarian follicles, is another member of the TNF family and also induces apoptosis in tumor cells. This ligand also binds to DcR1 and DcR2 receptors. DcR1 acts as a TRAIL neutralizing decoy receptor. The cytoplasmic domain of DcR2 activates NF-κB and leads to the transcription of genes that play a role in interfering death signaling pathway or enhancing inflammation [59] (Figure 1).

In the following, we will examine apoptosis in polycystic ovary cancer. Polycystic ovary syndrome (PCOS) is a common endocrine disease in females. PCOS patients reduce ovarian granular cells, which may be associated with cell apoptosis. PCOS, also known as ovulation disorder can cause infertility in females and menstrual dysregulation in patients. Patients with PCOS may develop into hyperandrogenism, high luteinizing / follicle stimulating hormone levels, or hyperinsulinemias. There are large amounts of small follicles exist in the ovaries of PCOS patients, but they do not have mature follicles due to hypothalamic-pituitary-ovarian dysfunction and dysregulation of ovarian function. Cellular apoptosis plays an important role in follicular growth in oocyte production, oocyte destruction, follicle selection, or even follicular atresia. In reproductive-age females, apoptosis occurs mainly in follicular granule cells, indicating a possible dysregulation of cell apoptosis in PCOS individuals. There is an active process during follicular granule apoptosis that is associated with the caspase family and the Bcl-2 family [60]. Granular cell apoptosis is regulated by apoptotic receptors, including Fas / Fas-L, in addition to the Bcl-2 family. Based on studies, it was observed that changes in apoptosis-related proteins in PCOS patients lead to decreased Bcl-2 expression as well as high expression of Fas, Bax and Fas-L in the antral follicles of PCOS patients. Increased expression of Fas and Fas-L in follicular granule cells indicates the cell activation and gradual apoptosis. Follicular granule cells of PCOS patients show abnormal differentiation, as well as enlargement of lateral granular cells at the side of oocytes with filling of the endoplasmic reticulum in the cytoplasm, which indicates the ability of these granule cells to synthesize hormones. In addition, these patients also have dysregulation of FSH-granular cells, which causes apoptosis of follicular granule cells and the inability of androgens to form estradiol. This process leads to an increase
Ahmadifar et al.
in the level of androgens in the body, impairing the follicle selective advantage, as well as the accumulation of small follicles to form cysts inside the ovary. In PCOS patients, these data suggest that therapeutic targeting of apoptosis could be a novel approach for treating patients with PCOS [61].

miRNA regulators

Micro Ribonucleic acids (microRNAs) are evolutionarily protected ribonucleic acids that are non-coding and have a length of about 18-25 nucleotides. They can control the expression of genes by breaking down mRNAs or inhibiting their translation. They do this by attaching to the untranslated region at the end of mRNAs. microRNAs (miRNAs) are regulatory, non-coding molecules that are encoded by plants, animals, and some viruses. These molecular structures can play a role in controlling physiological processes such as apoptosis, hematopoiesis, tissue differentiation, and brain morphogenesis, as well as in pathological processes such as oncogenes or tumor inhibitors called oncomir (by inhibiting the expression of target-dependent genes Cancer). Oncomirs are present in malignancies of various tissues and are often found in regions of the genome that have been deleted, duplicated, or mutated. Therefore, it can be predicted that mutations in them can lead to cancer. The identification of microRNAs and their target molecules have provided a clear horizon for identifying the pathways that lead to cancer [62]. MicroRNA expression changes cause tumors to arise by lowering the expression of key genes involved in cell proliferation and survival. MicroRNAs' placement in cancer-related genomic areas or fragile regions, on the other hand, can have a big impact on how they're expressed in tumor cells. It is noteworthy that epigenetic agents can inactivate microRNA by hypermethylation or histone modification, while microRNA itself is a regulatory factor for these factors [63].

RNA polymerase II / III transcribes miRNA genes and binds them to pri-miRNA. After that, Drosha transforms them into pre-miRNA. They then move to the cytoplasm, where Dicer converts them to adult miRNAs. Furthermore, miRNAs attach to the 3'UTR region of target genes, inhibiting gene transcription completely or partially. Dicer is a cytoplasmic RNase III enzyme that is essential for the processing of short regulatory RNAs, such as miRNAs (Figure 2). The importance of post-transcriptional gene regulators like
Ahmadifar et al.
Dicer and its products (miRNAs and siRNAs) in female fertility has been demonstrated as the function of Dicer and its products (miRNAs and siRNAs) in the female reproductive system has been explored. miRNAs regulate gene expression at the post-transcriptional level by mRNA deadenylation, suppression of translation, or miRNA-mediated mRNA degradation [64]. Fertilized oocytes and oocytes contain 10-15-fold higher levels of Dicer transcripts more than other cells or tissues and are one of the few known mammalian cells or tissues in which Dicer expression is regulated. Dicer transcript expression remains constant during follicular growth and in bud vesicles and metaphase II stages in growing oocytes. The amount of Dicer messenger RNA is lowered by around half after fertilization in the two-cell embryo and remains low through the blastocyst stage. The complete expression of miRNA is reached at the same time in the adult oocyte as well as in the unicellular zygote before dividing into two cells. Post-transcriptional gene regulation by microRNAs plays a key role in tissue formation and differentiation. Dicer1 ribonuclease III for the synthesis of mature, plays an important role in follicular cell development through the different regulation of gene expression.

Expression of TUBB8, SOX9 and BCL2 genes
Ovarian follicular growth and ovulation include selection of a dominant follicle, reactivation of oocyte meiosis, rupture of the follicle wall, cumulus-oocyte expansion, and tissue remodeling to form the corpus luteum [65]. They are microRNA-controlled mechanisms that are complicated and carefully regulated (miRNAs). In the formation of folliculogenesis, miR-29a, miR-30d, and miR-224 play a role. MiRNAs regulate post-transcriptional gene regulation, which is important for tissue development and differentiation. MicroRNAs, which affect reproductive endocrine activities such as oocyte maturation and folliculogenesis by repressing or degrading transcription, may play a crucial role in maintaining gene expression stability. MicroRNAs regulate the operation of gonadal somatic cells, granulosa cells, and cumulus cells, and hence play a role in steroid production. In the ovary, microRNAs are the most common type of short RNA. These miRNA molecules play a role in the ovary's gene control [66].

Several properties of miRNAs, including their stability and tissue character, make them suitable for their relative detection and measurement in a variety of tissues. Changes in miRNA expression can have a detrimental effect on the outcome of
Ahmadifar et al. Expression of TUBB8, SOX9 and BCL2 genes

various cellular activities regulated by the product of these genes, such as oocyte growth and maturation. Because these non-regulatory genes may be linked to different pathways such as lipid metabolism, insulin signaling, and oocyte maturation, which have been shown to be related to PCOS, it has been speculated that different expressed miRNAs may inhibit follicular growth. Specific metabolic disorders associated with PCOS have been shown to target specific miRNAs of important ovarian molecules such as progesterone receptor (PGR), aromatase (CYP19A1), follicle-stimulating hormone receptor (FSHR), and the FYN pathway. In humans, miRNAs have been shown in wall granulosa cells (cumulus versus mural granulosa cells), patients with polycystic ovary syndrome (PCOS), and cumulus cells due to oocyte nuclear maturity [67].

miR-132 and miR-212 play important roles as post-transcriptional regulators in granulosa cells. C-terminal binding protein 1 (CTBP1) is the target of miR-132. The gene product acts as the corepressor of nuclear receptor genes. Deletion of miR-132 and miR-212 reduces CTBP1 protein levels but does not alter mRNA levels. The family (let-7 family, miR-21, miR-99a, miR-125b, miR-126, miR-143, miR-145, and miR-199b) have the largest population of miRNA in the ovary. Bioinformatics prediction, screening and gene ontology analysis, the main target miRNA gene in mammalian ovary and has identified several biological processes and pathways or molecular networks, including cell cycle regulation, cell death, cell-to-cell signaling, cellular growth, development and proliferation, endocrine system disorders and various pathways of ovarian function. miRNAs may play a role in altering gene expression and hormone production in granulosa cells after FSH exposure [68]. Aromatase expression (CYP19A1) and thus E2 production by granulosa cells is post-transcriptionally downregulatory by miR-378. miRNA may play a key role in fine-tuning the gene expression cascade, leading to ovulation processes, luteal cell differentiation, and corpus luteum (CL) function in mammalian ovaries. The expression of specific genes involved in ovarian folliculogenesis and steroidogenesis may be directly regulated by miRNA expression in granulosa cells. In PCOS patients, miR-132 and miR-320 are significantly less expression. miR-132, miR-320 and miR-520c-3p regulate E2 concentration. The expression of miRNAs in PCOS patients was compared with that
of controls via microRray and qPCR, and the findings showed that miR-30c, miR146a and miR-222 were significantly increased in PCOS patients [69].

Insulin receptor substrate2, synaptogamin 1, and interleukin 8 were all shown to have considerably lower expression in women with PCOS. Insulin receptor substrate2 deficiency can cause estrous cycle disruption, infertility, and insulin resistance. In late follicular and ovulatory stages, interleukin 8 is involved in steroid synthesis. All three have roles in carbohydrate metabolism and cell function, cell-cell communication, and steroid synthesis, all of which are linked to the PCOS phenotype. Alterations in miRNAs involved in the steroid and insulin signaling pathways could be linked to the pathogenesis of PCOS and related illnesses, and could be the subject of future research.

Expression of TUBB8, SOX9 and BCL2 genes

miRNAs 483-3P, 363-3P and 4284 have an inhibitory roles in some stages of the transforming growth factor (TGF) -β signaling pathway through changes in Smad3 and Smad4 (a family of structural proteins) [70]. TGF signaling is inhibited by miRNA-24, by suppressing the production of Smad proteins. Growth factor signaling conversion is known as E2 releasing factor and regulation of miRNA-24 at E2 concentration can be explained by a mechanism in which overexpression of miRNA-24 reduces TGF-β signaling. As a result, E2 secretion is inhibited. TGF-growth factors have a critical role in folliculogenesis and oocyte growth [71].

Scientists can better understand the mechanisms of gene control that permit successful reproduction by identifying specific miRNAs or siRNAs in each female reproductive tissue. Given the main function of regulating miRNAs in the stability of gene expression, understanding the basic mechanisms of how ovarian follicle miRNAs are regulated and their specific target genes identified and their function can lead to the prevention development and treatment strategies by regulating specific target genes, which related to reproductive disorders [66,67].

Structure and expression of TUBB8, SOX9 and BCL2 genes

TUBB8

Microtubules are made up primarily of tubulin. Microtubules, such as TUBB8, are dynamic polymers made up of alpha-tubulin polypeptide and beta-tubulin polypeptide heterodimers. TUBB8 is only found in the oocyte and early embryo, where it is responsible for nearly all of the beta-tubulin produced. This gene encodes a special β-tubulin isotype that is the main
Ahmadifar et al.

constituent of the oocyte and early embryo spindle and only exists in primates. Mutations in TUBB8 were previously discovered to induce oocyte maturation arrest. Oocyte maturation, fertilization, and early embryonic developmental arrest have all been linked to a genetic defect in the VIII class of the TUBB8 gene. The TUBB8 gene has four coding exons, according to Feng et al. (2016) [72]. Hartz (2016) mapped the TUBB8 gene to chromosome 10p15.3 based on an alignment of the TUBB8 sequence with the genomic sequence (GRCh38). Heterozygous or de novo mutations in the TUBB8 gene are thought to cause MI arrest predominantly through dominant-negative effects, while some arrest occurs during early embryonic development. However, homozygous or compound heterozygous mutations may lead to fertilization failure (MII arrest) and early embryonic developmental arrest. According to previous reports, mutations in TUBB8 account for approximately 30% of the individuals with MI oocyte arrest. These findings suggest that different mutations might result in different structural abnormalities and might alter TUBB8 interactions with kinesin or TUBB8 binding to other microtubule-associated proteins. Although multiple

Expression of TUBB8, SOX9 and BCL2 genes

TUBB8 mutations were found in infertile patients, effective treatment has not been reported [73]. A novel heterozygous mutation in TUBB8 was discovered in some studies to be responsible for mitosis division defects and early embryonic development arrest. This mutation interferes with mitotic spindle assembly and chromosome arrangement, causing early embryonic development arrest rather than oocyte maturation and fertilization. It was also found that the entrance of wild-type TUBB8 cRNA into mutant TUBB8-expressing oocytes can successfully rescue the developmental defects of the resulting embryo and produce full-term offspring. Studies showed that TUBB8 mutants are responsible for a large proportion of oocyte maturation defects in infertile women. TUBB8 and TUBA1C were found to have the highest expression in PCOS oocytes among the genes involved in the microtubule-based process, according to one study. TUBB8 and TUBA1C overexpression in oocytes from women with PCOS was confirmed by immunofluorescence (IF). It has been found that TUBB8 plays an important role in meiotic spindle assembly and maturation in human oocytes and mutations in TUBB8 leads to oocyte
maturation arrest. Pathogenic missense TUBB8 mutations affect microtubule dynamics and impair oocyte meiotic spindle assembly via dominant-negative effects [71, 74].

Oocyte maturation and subsequent mitosis are important periods for normal embryo development. Early embryonic arrest is frequently caused by aneuploidy caused by abnormal spindle assembly. Mitosis was more sensitive to the effect of spindle assembly than meiosis, according to oocyte-injected mutant TUBB8. In mice and humans, two bipolar spindles form in the zygote and then assemble the maternal and paternal genomes independently, according to new research. This spindle assembly mechanism provides a potential rationale for zygote spindles being sensitive to microtubule gene mutation and shows the differences between the mechanisms of mitosis and meiosis during oocyte and zygotic transformation. This TUBB8 mutation may provide a unique perspective on the differences between meiotic and mitotic spindle assembly in the maternal-to-zygotic transition in mammals [74].

**SOX9**

Sry-related high-mobility group box (SOX) proteins are a wide family of transcription factors that control a range of developmental and tissue-specific processes through a homologous high-mobility group (HMG) DNA-binding domain. SOX9 stimulates the expression of anti-Müllerian hormone in the developing gonad, which plays a crucial role in determining male sex (AMH). In humans, the SOX9 gene is found on the 17q24 chromosome. SOX9's HMG domain, which belongs to the SoxE subgroup, forms an L-shaped complex in the minor groove of DNA that induces considerable bending at the consensus-binding motif (A/TA/TCAAA/TG) [75, 76].

SOX9 controls the cartilage collagen gene COL2A1 in the testis and a range of other tissues, including chondrocytes. The SOX9 transcription factor controls the differentiation of a range of cell types in vertebrates. With many tissue-specific enhancers, the SOX9 gene is big and complex. Individual enhancers regulate SOX9 expression in chondrocytes, Sertoli cells, and cranial neural crest cells. Depending on whether the mutation occurs in the coding or enhancer regions, human SOX9 mutations can cause the Campomelic Dysplasia syndrome or isolated clinical features. In hair follicle stem cells and chondrocytes, where SOX9
Ahmadifar et al. binds to super-enhancers, chromatin immunoprecipitation has assisted to identify SOX9 regulation of target gene expression at the genome-wide level. The proximal binding of SOX9 to the promoter regulates basal cell activity, whereas distal enhancers control cell type specificity. [75, 77].

The SOX9 gene has been implicated as an oncogene in a variety of cancers, although it might potentially function as a tumor suppressor. SOX9 is overexpressed in a wide spectrum of human cancers, and its expression has been linked to tumor development and clinical data, indicating that it plays a key role in tumorigenesis. In combination with other oncogenes, SOX9 interacted with different transcription factors and demonstrated multiple pro-oncogenic characteristics, including proliferation promotion, senescence inhibition, and neoplastic transformation [78].

SOX9 has varied expression levels and functions in normal ovarian development compared to other tissues. SOX9 is not expressed in early pre-antral follicles during follicular development, but it is expressed in the cells surrounding the growing follicles. The formation of collagen or laminin fibers, which make up the follicular lamina, is regulated by these genes. The function of SOX9 in ovarian cancer is little understood [79]. Despite this, SOX9 expression was found to be higher in human Sertoli tumor biopsies when coexpressed with BCL-2 and Ki-67, with the latter being less expressed in well-differentiated cells. This indicates that the stage of tumor differentiation has an impact on apoptotic and proliferative capabilities. Hypoxia is also known to enhance the expression of Tubulin Beta 3 (TUBB3) via HIF-2 and SOX9. High expression of these genes has been linked to a lower overall survival time in women with ovarian cancer [78]. According to one study, the embryonic pathway of prostaglandin D synthase (Pgds) / SOX9 is expressed at both the RNA and protein levels in many types of human ovarian tumors, making Pgds and SOX9 diagnostic indicators for ovarian cancer. In this study, using ovarian cancer cell lines, first, the expression of Pgds / SOX9 pathway components in these cells, and second, treatment of these cells with prostaglandin D2 (PGD2) can inhibit their growth through the DP1 receptor and cause apoptosis [80].

SOX9 plays a critical role in the development of pancreatic, gastric, and prostate cancers. SOX9, on the other hand,
Ahmadifar et al.
is involved in the progression of bladder and colorectal cancer, while it is linked to metastasis in breast, gastric, pancreatic, and colorectal cancer. Furthermore, SOX9 is clinically important because it may play a role in cancer diagnosis, prognosis, and treatment. This is because, depending on the stage, location, and aggressiveness, its level of expression and location could be cytoplasmic or nuclear. As a result, it could be used as a biomarker [76, 78].

**BCL2**

B-cell lymphoma 2 (BCL2) is a proto-oncogene that was first cloned as a result of its ongoing involvement by t (14;18) (q32; q21) in lymphoma, where its transcription is driven by the immunoglobulin heavy chain (IGH) gene enhancer on chromosome 14q32, resulting in constitutive expression of BCL2 in B-cell clones. Unlike many other oncogenes, BCL2 promotes cell survival by preventing apoptosis, or programmed cell death. The human BCL2 gene has three exon structures with untranslated first exon, a facultative 220 bp intron I, and a large 370 kb intron II [81]. The native BCL2 gene has a long 3′ untranslated region and two distinct promoters, P1 and P2. The major promoter region, P1, is a TATA-less, GC-rich promoter containing multiple transcriptional start sites and located 1386-1423 bp upstream of the translation start site. When an alternative promoter is used, mRNAs with exons II/III or I/II/III are produced. In B-cells, the t (14;18) translocation results in 4.2-7.2 kb BCL2-IGH chimeric mRNAs with different BCL2 5′ exons and different IGH 3′ untranslated regions. The t (14;18) doesn’t disconnect the BCL2 open reading frame, however, unsuitably high levels of BCL2-IGH mRNAs are present. The native BCL2 and BCL2-IGH fusion mRNAs represent the same 2.5-hour short half-life. BCL2 is located on chromosome 18q21.33 in a telomere to centromere orientation normally. The molecular consequence of the t (14;18) juxtaposes of the BCL2 gene next to IGH locus on the der(14) chromosome, in the same transcriptional orientation as the IGH gene [82].

The BCL2 gene encoding a 26-kD protein with 239 amino acids and a single highly hydrophobic domain at its C-terminus, allowing it to be found primarily in the mitochondrial outer membrane, as well as the nuclear envelope and endoplasmic reticulum membrane. The main function of the BCL2 protein is to keep the mitochondrial membrane intact by
Ahmadifar et al. preventing cytochrome c release and subsequent binding to the apoptosis activating factor-1 (APAF1) protein [83]. All four BCL2 homology (BH) domains are present in the protein (BH1 to BH4). BH1, BH2 and BH3 organize the hydrophobic cleft through which the protein interacts and forms homo- and heterodimers with the pro-apoptotic members of the BCL2 family of proteins. By inhibiting apoptosis, BCL2 improves cell survival kinetics. As a result, it prevents the cell from engaging in suicidal activities that normally necessitate the production of ATP, new RNA, and protein. It also causes a variety of cellular ultrastructural changes, such as cell shrinkage, nuclear fragmentation, and DNA degradation. BCL-2 proteins regulate mitochondrial outer membrane permeabilization (MOMP), which leads to the irreversible release of intermembrane space proteins, caspase activation, and apoptosis. This family consists of 25 pro-apoptotic and anti-apoptotic members which interact to maintain a balance between newly formed cells and old dying cells. BCL2 family proteins share up to four BCL2 homology domains (BH1, BH2, BH3, & BH4) [84]. The anti-apoptotic proteins, BCL2, BCL2L1 (BCL-XL), BCL2L2 (BCL-W), MCL1, BCL2A1

Expression of TUBB8, SOX9 and BCL2 genes (A1/BFL-1), share homology within four domains (BH1-4). BCL2 family proteins are important regulators of cell death, regulating apoptosis, necrosis, and autophagy, among other cell death mechanisms. They are important regulators of the mitochondrial apoptosis pathway. Pro-apoptotic proteins like BAX, BAK1, and BOK all have BH1-3 domains, whereas BH3-only proteins like BCL2L11 (BIM), BAD, and BID only have the BH3 domain. BH3-only proteins are inactive or present at low levels in the cell in normal circumstances. However, in the presence of apoptotic stimuli, post-translational modifications activate BH-only proteins, or their expression is increased. BAX-BAK oligomerization is induced by the stimulation of BH3-only proteins. BAX and BAK directly cause MOM permeabilization after oligomerization, which is an important step in apoptosis. Anti-apoptotic BCL2 proteins neutralise pro-apoptotic BH3-only proteins, preventing them from inhibiting BAX-BAK activity and MOM permeabilization. A major factor in determining whether cells undergo apoptosis in response to cell stress is the balance between pro-survival and pro-death BCL-2 proteins [85] (Figure 3).
Figure 1: Pro-apoptotic proteins, prevents c-Myc induced apoptosis, and also blocking the release of cytochrome-c in mitochondria. This compound prevents the interaction of Apoptotic protease-activating factor 1 (Apaf-1) with caspase-9. Bax and Bok initiate By binding and inhibiting upstream caspase-9 and the downstream caspase -3 and -7 (IAPs), inhibits both intrinsic and also extrinsic apoptotic pathways. TNFRI forms a cytoplasmic TRADD complex which contains FAS-associated death domain (FADD) that activates caspase-8 by stimulating procaspase-8, leading initiation of an apoptotic signaling cascade. A member of the TNF family, Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in tumor cells. TRAIL and its receptors are expressed in growing, atretic and antral ovarian follicles.
Figure 2. RNA polymerase II transcribes a primary miRNA (pri-miRNA) and Drosha in complex converting it into pre-miRNA. These stages take place in the nucleus. Then Dicer processes pre-miRNAs after they have been transported from the nucleus to the cytoplasm by Exportin5 and RANGTP. When pre-miRNA converted to mature miRNA, it binds to a RNA-induced silencing complex (RISC); it causes mRNA degradation or translational repression. As a result, either translation is inhibited or the mRNA target is degraded.
Figure 3. BCL2 especially by preventing apoptosis increases the survival kinetics of the cell. Accordingly, it blocks processes in the cell from going into suicidal activities that generally require ATP, new RNA, and protein synthesis. The BCL-2 family of proteins controls cell death primarily by direct binding interactions that regulate mitochondrial outer membrane permeabilization (MOMP) leading to the irreversible release of intermembrane space proteins, subsequent caspase activation and apoptosis. In the existence of apoptotic stimuli, the post-translational modifications activate or increase the expression of BH-only proteins. The stimulation of BH3-only proteins induces BAX-BAK oligomerization. The pathway of promoting apoptosis by Bax protein may be related to the anti-apoptotic protein Bcl-2, which can cause Bax to lose its pro-apoptotic effect.
Expression of BCL2 and Prognosis

Multiple studies have shown that high levels of BCL2 gene expression are negative risk factor associated with severity of malignancy. Elevated expression of BCL2 in AML was shown to be associated with poor clinical response to chemotherapy. Within a specific subgroup of DLBCL, BCL2 expression is negatively correlated with overall survival. Furthermore, high BCL2 expression has been linked to a poor prognosis in melanoma, breast, prostate, small cell lung, colorectal, and bladder cancers in several studies [86]. Higher BCL2 expression has been linked to resistance to chemotherapy and radiation in other studies. BCL2 expression is widespread in immature tissues before birth, but it becomes highly restricted as the maturation. BCL2 is widely expressed in immature B-cells and memory B cells, but it is temporarily suppressed in germinal center B-cells, possibly due to BCL6 repression. Its expression levels are reduced in motor neurons and pre-B cells that are preparing to differentiate. BCL2 is upregulated in nearly half of all human cancers, which is consistent with its function as an apoptotic regulator. The majority of small cell lymphoma such as chronic lymphocytic leukemia (CLL), marginal cell lymphoma, and mantle cell lymphoma, over-express BCL2, although less than 5% of those patients have detectable BCL2 gene rearrangement. In nearly all patients with acute lymphocytic leukemia and mostly in acute myeloid leukemia Increased expression of BCL2 have found. Ovarian, neuroblastoma, bladder, colorectal, and some head and neck cancers have all exhibited high expression of BCL2 [87].

The Fas system and the Bcl-2 family are normally considered the regulation of apoptotic signaling in the ovary. Members of the Bcl-2 family of proteins is one of the main regulatory proteins. Many scholars believe that the key of regulating factor in cell apoptosis is the inhibitor of apoptosis gene Bcl-2 which affect the apoptosis by affecting intracellular information conduction. Bax protein is a related protein homologue of Bcl-2 and acts as an antagonist of Bcl-2. Studies have shown that Bax expression in tissues and organs is more widespread than that of Bcl-2 in mice, and it is also
Ahmadifar et al.

expressed in the male testes and female ovaries. The pathway of promoting apoptosis by the Bax protein might have a relation with the Bcl-2 (anti-apoptotic protein), which can cause Bax efficiency of losing its pro-apoptotic. So if some kind of biological active ingredient or drug could improve the expression of Bcl-2 mRNA and impede the expression of Bax mRNA, then it should have important value for delaying or reducing cell apoptosis [87,88].

Functional mechanism of gene expression

Gene expression (GE) is the synthesis of a functional gene product using information provided by deoxyribonucleic acid (DNA). Through the transcription process, which is part of the GE process, Ribonucleic acid (RNA) is synthesized from DNA. Cells have the ability to adjust the type and amount of GE. We first inspect the mechanism of gene expression [89]

Transcription

Transcription is the process by which a functional gene product (i.e., RNA) is synthesized. This product or transcript may be a protein precursor (via translation), a subunit for a larger molecule (e.g., ribosome) or a functional molecule in and of itself (e.g., miRNA). A transcriptome is the complete set of RNA transcripts that exist in a cell at a specific growth stage or physiological condition. Transcription includes some steps such as the unwinding of DNA, the appeal and binding of transcription factors. In addition, the function of the transcriptional machinery to make RNA from the DNA pattern. It is performed by RNA polymerases, which add a ribonucleotide to a growing RNA strand at the same time, according to the law of complementation of nucleotide bases [90]. In eukaryotes, transcription is performed in the nucleus by three types of RNA polymerases, each of which requires a special DNA sequence called a promoter to begin the process: The RNA polymerase I enzyme that only transcribes rRNA genes, The RNA polymerase II enzyme transcribes only mRNA precursor genes as well as some small RNAs and The RNA polymerase III enzyme transcribes only tRNA genes as well as some small RNAs [91].

In general, the transcription process consists of 3 steps. These steps include Initiation, Elongation and Termination. Initiation is the beginning of transcription. It happens when the
enzyme RNA polymerase binds to promoter which is a region of a gene. DNA in this time will be signaled to unwind and the enzyme can “read” the bases in one of the DNA strands. Now the enzyme is ready to create a strand of mRNA with a complementary sequence of bases. Elongation is the addition of nucleotides to the mRNA strand. RNA polymerase reads the unwound DNA strand and by complementary base pairs builds the mRNA molecule. Process of bounding newly formed RNA to the unwound DNA takes a brief time. While this process happening, an adenine (A) in the DNA binds to an uracil (U) in the RNA. When RNA polymerase crosses a stop (termination) sequence in the gene termination occurs which is the ending of transcription. The mRNA strand is complete, and it detaches from DNA [92] (Figure 4).

Regulatory regions through DNA and transcription factors are the elementary units involved in the transcription process. RNA is a linear polymeric molecule composed of a ribose sugar, a phosphate group, and nitrogenous bases (i.e., adenine, guanine, cytosine and uracil). Researchers determine GE in the cells by calculating different kinds of RNA (i.e., coding and non-coding). RNA transcripts are normally categorized as protein non-coding or coding. The human genome includes about 20,000 protein-coding genes and at least the same number of non-coding RNA genes. Coding genes synthesize messenger RNAs (mRNAs) that produce protein products through the translation process. mRNA is different from other types of RNA because of the polyadenylate (poly[A]) at the end of their transcript. One exception is histone mRNA that has a conserved 3’ stem loop instead of a poly (A) tail. Non-coding RNAs make up the majority of RNA species in the cell. There are two major types of non-coding RNAs: the small noncoding RNAs and the long noncoding RNAs. Small non-coding RNAs include microRNA (miRNA), transfer RNA (tRNA) -derived small fragments, P-element Induced Wimpy (PIWI) protein-interacting RNA, small nucleolar RNA and small interfering RNA. Long non-coding RNAs include promoter associated long RNA, transcribed ultraconserved regions, tRNA, circular RNA (circRNA), small nuclear RNA (snRNA), pseudogenes and antisense RNA. Ribosomal RNA (rRNA) is the most abundant type of RNA in the intracellular matrix and belongs to neither small nor long classes without RNA
Ahmadifar et al. 

encoding. Both tRNA and circRNA have principal roles in protein synthesis. Each tRNA carries the specific amino acid which needed to expand the protein during mRNA translation. On a ribosome, tRNA binds to mRNA along a three-nucleotide anticodon sequence. CircRNAs have covalent bonds between the 3' and the 5' ends and are involved in transcription, regulation of RNA splicing after transcription, and sequestration and suppression of miRNA activity. miRNA regulates the expression of several genes, while regulation of miRNA function by circRNA highlights the increased complexity in non-coding RNA regulatory pathways [93].

**Processing**

In prokaryotes, the direct product of transcription from genes (genes that produce proteins) is mRNA, but in eukaryotes, a pre-mRNA is first made that will be converted to mRNA after a series of operations. This primary RNA transcript is then modified to transform it into a full-grow messenger RNA (mRNA) which can be used in translation. The mRNA undergoes splicing to remove the non-coding parts of the transcript (introns) so that only the coding sections (exons) remain [89,94].

**Expression of TUBB8, SOX9 and BCL2 genes**

**Non-coding RNA maturation**

Non-coding regions of the RNA (ncRNA) can first be transcribed as precursors and then further processed. In the case of ribosomal RNAs (rRNAs), they are frequently transcribed as a pre-rRNA comprising one or more rRNAs. Pre-rRNA is broken down and modified at specific sites by approximately 150 different small species of RNA confined to the nucleus, called snoRNAs [89,95].

**RNA export**

In eukaryotes, most mature RNAs must leave the nucleus and enter the cytoplasm. While some RNAs role in the nucleus, many RNAs through nuclear pores are transported into the cytosol. The export of RNAs requires the association with certain proteins known as exportins. Sometimes some RNAs need to be transported to specific locations in the cytoplasm, such as synapses. Then, they bound to RNA by motor proteins that bind through linking proteins to specific sequences (called "zipcodes") [89,96].

**Translation**

Ribosomes in this process in the endoplasmic or cytoplasm reticulum synthesize proteins after the process of transcribing DNA to RNA in the cell
nucleus. For some genes, the end product is RNA produced in the previous stages, but for many, like mRNAs, this is not the last step. mRNAs can carry the code of one or more protein sequences. In each mRNA sequence, genetic information is stored by ternary codes called codons. In the cytoplasm space, for each of these codons, there is a tRNA with a complementary ternary which carries with it a specific amino acid. These amino acids are then linked together by a ribosome as well as a sequence of codons on the mRNA to form a protein sequence. Many protein sequences can be made from each mRNA strand [97] (Figure 5).

**Protein folding**

After the translation of mRNA sequence into a linear chain containing of amino acids, proteins are presented in the shape of an unfolded polypeptide or as a random coil. This polypeptide doesn't have any developed three-dimensional structure. The correct shape of three-dimensional structure is necessary for the function even though some parts of practical proteins might still remain unfolded. Chaperones enzymes help new proteins to get the 3-dimensional structure they need to function. The long chain amino acids are folded by enzymes called chaperones and found a three-dimensional structure. This three-dimensional structure creates the function and final shape of the protein. Helping protein folding is one of the main roles of the endoplasmic reticulum in eukaryotes [98].

**Regulation of gene expression**

The processes involved in GE regulation include transcription, a number of epigenetic processes and post-transcriptional modifications. Regulation of GE is specific for the cell itself and involves various complex biochemical processes that are necessary for the growth of the organism and the organism's ability to answer to changes in the environment. Understanding GE regulatory mechanisms is crucial [99].

**Regulatory regions**: Specific regions of DNA involved in GE transcription and regulation in eukaryotes include promoters, enhancers, silencer and insulators. Promoters and enhancers are the DNA regulatory regions which called cis-regulatory elements. These regulators form the cis-regulatory module (CRM), also called the transcription factor binding site (TFBS). Transcription factors bind to TFBS for regulating GE. Upstream of the transcription initiation
Expression of TUBB8, SOX9 and BCL2 genes

Ahmadifar et al.

site is the promoter region of a gene that typically, has a protected sequence (about 25 to 35 bp) contains TATA repeats motifs. Binding of the transcription factors to the promoter region of the gene, leads to facilitate the binding of RNA polymerase for starting the transcription. Although RNA polymerase catalyzes mRNA synthesis, by itself it is incapable of binding to DNA and initiating transcription at specific sites [100]. Initiation of transcription as like as the recruitment of RNA polymerase II requires general transcription factors. Six general transcription factors are known—TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH—each of which contains multiple subunits [101]. TWhat determines the transcription direction is the promoter sequence. The DNA strand that is transcribed is the sense strand. Transcription activation is done by the enhancer regardless of the location, distance or direction of the promoter. The enhancers contain multiple TFBS and can be transcribed into non-coding RNAs. These non-coding RNAs, together with a protein complex called cohesin, stabilize and facilitate transcriptional enhancer-promoter interactions. Silencers are areas of DNA that interact with enhancers. Transcriptional repression occurs through two types of silencers, silencer elements and negative regulatory elements (NREs) [102]. Silencers, which are an important part of the promoter region, determine the mechanism of transcription suppression.

An important basis for GE regulation is the interaction between the silencer and the enhancer and other transcription elements. Insulators are DNA sequences that protect an expression gene. Two types of DNA insulators sequences include the barrier element and the enhancerblocking element. The barrier element binds a protein complex that prevents DNA methylation. In contrast, the enhancerblocking element interferes with the interactions between the enhancer and promoter of DNA regions. enhancerblocking occurs when there is an insulator between enhancer and promoter. This insulator element prevents GE from activating the adjacent gene [102,103] (Figure 6).

Transcription factors: Transcription factors are the proteins which regulate and the cause of transcription. Less than 2000 transcription factors are controlling GE. Transcription factors have common structural patterns such as zinc finger, a leucine zipper and helix-loop-helix structures. Protein kinases activate inducible transcription factors to bind to
Ahmadifar et al.
target response elements. Increased serum levels of the hormone initially activate certain receptors at the cellular level. It then creates a chain of signaling pathways to activate transcription factors. It should be noted that any changes in hormone levels, expression of transcription factors or cell-surface receptors can alter GE levels. Same as an overexpression of c-MYC which lead to cancer, overexpression of these factors can lead to morphological changes [104].

**Epigenetic regulation:** Epigenetic regulation allows changes to be made in GE in response to the environment. Factors involved in GE epigenetic regulation include DNA methylation, histone alterations, or non-coding RNA expression. By DNA methylation of a CpG island in the promoter region of the gene which preventing transcription factors from binding to the site of the methylated promoter site lead to repress the GE. DNA hypermethylation can lead to the silencing of tumor represesor genes such as BRCA1, which leads to tumorigenesis [105]. In contrast, in some special cases, CpG islands can activate transcription. Histone modification is another means of regulating GE epigenetics. The structure that results from the binding of DNA to histones is called a nucleosome. Certain amino acids on the histone protein can be modified by acetylation, phosphorylation, or methylation. Histone modifications can affect GE through two mechanisms. First, histone modifications can lead to less compact DNA structure, which makes it more accessible for transcription. Second, proteins can bind to the modified amino acids on histone proteins and alter DNA transcription [106].

Non-coding RNAs play an important role in regulating transcriptional and post-transcriptional processes and finally, GE epigenetic regulation can be mediated by them. For example, miRNAs regulate GE by suppressing translation or promoting mRNA degradation. miRNAs can bind to mRNA molecules and inhibit protein synthesis. The expression of these miRNAs varies over time depending on changes in intracellular and external environments. Epigenetic mechanisms of gene expression are served by three distinct yet highly interrelated mechanisms. DNA methylation refers to the addition of a methyl group to the position of 5 cytosines in the field of CpG dinucleotides to define the "fifth DNA base". The nucleosome is composed of an octamer of major histone proteins. Posttranslational modifications of the
Ahmadifar et al. amino-tails of histone proteins and the density of these proteins per unit length of DNA can significantly affect the structure of chromatin and form a hypothetical "histone code". RNA-based mechanisms have also recently been shown to affect the higher-order structure of chromatin [107] (Figure 7).
Figure 4: Transcription is the process of making an RNA copy of a gene sequence. During initiation, RNA polymerase identifies a specific place on the DNA called a promoter site, upstream from the gene that will be transcribed, and then unwinds the DNA locally. The next phase of transcription (elongation) can begin once RNA polymerase is in place at the promoter. Elongation is the process through which the RNA strand gets longer. RNA polymerase will continue to transcribe until it gets signals to stop. Transcription will stop by a process called termination; while the polymerase transcribes a sequence of DNA known as a terminator, termination happens.
Figure 5: Translation is the process of protein synthesis. Translation can begin after the ribosome, mRNA and tRNA bind together. Then, amino acids are transferred to the ribosome by tRNAs and joined together to form a chain. When a stop codon in the mRNA (UAA, UAG, or UGA) enters the A site, the finished polypeptide released and translation ends so the small and large ribosomal subunits separate from the mRNA and from each other.
Figure 6. Regulatory reign: The basal transcriptional machinery assembles at a region of DNA that is immediately upstream from the gene which includes the transcription initiation site and TATA box (gene promoter). TFIIA, TFIIIB, TFIIID, TFIIE, TFIIF, and TFIIH are Six general transcription factors and contains multiple subunits. Transcriptional repressors bind to silencers, and activators bind to enhancers.

Figure 7. DNA methylation of a CPG island in the promoter region of the gene can repress GE by preventing transcription factors from binding to the site of the methylated promoter site. DNA binds to histone proteins and form a nucleosome. Certain amino acids on the histone protein can be modified by acetylation, phosphorylation, or methylation. Histone modifications can affect GE through two mechanisms. First, histone modifications can lead to less compact DNA
Ahmadifar et al.

Expression of TUBB8, SOX9 and BCL2 genes

structure, which makes it more accessible for transcription. Second, proteins can bind to the modified amino acids on histone proteins and alter DNA transcription. Eventually, GE epigenetic regulation can be interceded by non-coding RNA expression. The nucleosome is composed of an octamer of main histone proteins.

Post-transcription processes: These biological processes are common to most eukaryotic cells, involving chemical modifications of the primary RNA transcription product to produce a functional mature RNA molecule. All regulatory mechanisms that control gene expression after transcription initiation are known as post-transcriptional control [108]. Post-transcriptional processes convert uncoding RNA into a functional gene product and prepare the mRNA for translation. In eukaryotes, there is a gene in the distance between the promoter and the terminal site whose main parts are called exons and the distances are called introns. Post-transcriptional processes include 5’pre-mRNA capping, removal of intron sequences from RNA by splicing, alternative pre-mRNA splicing, addition of a poly (A) tail to pre-mRNA, gene fusion transcript processing and stability mRNA modulation. Among these processes, deletion of intron sequences from RNA by splicing, alternative splicing, and the process of gene fusion transcript processing can affect GE regulation. The coding region of a gene is called an exon [109]. On average, a human gene contains 10-15 exons. At the beginning of transcription, the sequence of exons and introns is completely copied into the original RNA. After making changes such as shortening the initial RNA length, the molecule is sent to the cytoplasm for translation. Copies of introns need to be removed for this shortening. Splicing of pre-mRNA sequences is catalyzed by RNA protein complexes called small nuclear ribonucleoprotein particles (snRNPs). The RNAs found in snRNP are long, noncoding RNAs. Alternative splicing occurs when a gene causes different transcripts. Alternative splicing of pre-mRNA transcripts can lead to changes in GE. Through alternative splicing, exon sequences can form different compounds that encode different versions of the protein. Thus, mRNA transcripts of a gene can differ as a result
Expression of TUBB8, SOX9 and BCL2 genes


Ahmadifar et al.


Expression of TUBB8, SOX9 and BCL2 genes


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Ahmadifar et al.


Expression of TUBB8, SOX9 and BCL2 genes


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Ahmadifar et al.


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Expression of TUBB8, SOX9 and BCL2 genes


Ahmadifar et al.  


Expression of TUBB8, SOX9 and BCL2 genes  


Ahmadifar et al.


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Expression of TUBB8, SOX9 and BCL2 genes


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