

Review

DNA Methylation and microRNA patterns are in association with the expression of BRCA1 in ovarian cancer

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Abstract: Ovarian cancer is the sixth most prevalent cancer in women and is considered the most lethal gynecological malignancy. It can be inherited as a familial disease but also has a strong spontaneous occurrence. Although the disease is associated with genome instability brought on by genetics and environmental factors there is evidence that mutations in the gene encoding for the breast cancer type 1 susceptibility protein (BRCA1) or its down-regulation are involved in its development. Down-regulation of BRCA1 expression by hypermethylation of its promoter may account for some cases of ovarian cancer but this does not explain the cause of the majority of the disease. This review explores the role of BRCA1 promoter hypermethylation and micro-RNAs (miRNA) involved in the regulation of BRCA1 and their role in ovarian cancer development as well as some of the exciting discoveries which could lead to targeting miRNA with a view to restoring BRCA1 expression in diseased tissues.

Key words: Ovarian cancer, BRCA1, Hypermethylation, Micro-RNA.

Introduction

Epigenetic factors including DNA methylation and micro-RNAs (mi-RNAs) play crucial roles in the transformation of normal cells to malignancies (1). DNA methylation is a cellular mechanism which regulates gene expression (2). Methylation is performed by covalent adhesion of a methyl (CH₃) group to a cytosine (3). The added methyl groups interfere with the major groove of DNA and suppress transcription (3). DNA methylation occurs within CpG islands of genes including housekeeping, onco and tumor-suppressor genes and regulates their transcription in all mammalian cells (2). It has been reported that abnormal methylation leads to alterations in the expression of several genes, including oncogene and suppresser genes, which regulate mitotic division (4, 5). Thus, it has been hypothesized that DNA methylation may play a key role in the development of cancers.

Micro-RNAs (mi-RNA) are small RNAs which may regulate gene expression at the translational level (6). Because of their broad range of targets, including genes involved in cellular division, they are considered as potential regulators of tumorigenesis.

Ovarian cancer (OC) is the sixth most prevalent cancer in women and is considered the most lethal gynecological malignancy (7). Epidemiological studies revealed that the prevalence of OC varies extensively among ethnic groups and within different geographic regions (7). Despite the recent information regarding the pathophysiology of ovarian cancer, our knowledge regarding the etiology of OC is poor. It has been suggested that altered DNA methylation in onco/suppressor genes may

be a major factor responsible for malignancies derived from ovarian cells (8). Previous investigations demonstrated that BRCA1 (breast cancer 1) acts as a tumor suppresser gene and plays significant roles in the inhibition of mitotic division in several human cell lines and alterations in its expression may be associated with OC (9). Based on the important roles played by DNA methylation and mi-RNAs on regulation of gene expression, it has been hypothesized that alterations in BRCA1 gene DNA methylation or expression of some specific mi-RNAs may be associated with reduced BRCA1 expression which is accrued in OC. This review focusses on two aspects of BRCA1 regulation, the first is the transcriptional control of the gene by promoter methylation, and the second topic discusses the role of microRNAs in the control of BRCA1 expression. Indeed, there may be duality of function in which microRNAs may control transcription of BRCA1 through promoter methylation and in addition, microRNAs may also control translation of BRCA1 transcripts.

DNA methylation

DNA methylation is an epigenetic event which

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can regulate gene expression at the transcriptional level (10). Methylation is a process in which a methyl group is added or replaced on a substrate. The structure of methyl group consists of an alkyl derived from methane and contains a three hydrogen bonded carbon atom (CH_3) (11). In biological systems, methylation is facilitated by enzymes, especially DNA methyltransferases (DNMTs), and can result in changes of several cell functions including regulation of transcription, protein function and metabolism of RNA (12). DNMT1, DNMT3a and DNMT3b are the main enzymes which participate in establishment and maintenance of DNA methylation. It appears that DNMT1 is the main enzyme for the maintenance of established patterns of DNA methylation (11). The function of DNMT3a and 3b is to establish *de novo* DNA methylation patterns (11).

DNA methylation occurs at CpG sites of human genes (13). However, it appears that embryonic stem (ES) cells are exceptions to this general rule and a significant amount of methylation also occurred in non-CpG contexts (14). Interestingly, approximately 1.5% of human DNA is methylated at CpG sites (13) while CpG islands are located in the 56% of the promoters of mammalian genes and their methylation leads to transcriptional suppression of their downstream gene (15). Interestingly, 70% to 80% of cytosines in CpG sequences are methylated (15). When a gene is targeted for transcription, demethylation will occur by removal of methyl groups (CH_3) from CpG islands (15). Methylation is an important mechanism for the development and differentiation of tissues, as previous studies demonstrated that DNA methyltransferase knockout mice died in the morula stage (16). Interestingly, recent data raised the hypothesis of interactions of environmental factors and DNA methylation. Accordingly, it appears that DNA methylation is altered by several environmental factors such as stress, infection, obesity, free radicals and so on (17, 18) which are considered as risk factors for cancers. Therefore, it may be possible that DNA methylation provides a plausible link between environmental factors and the incidence of cancers.

Introducing mi-RNA

Mi-RNAs are short 20-22 nucleotide (nt) RNA molecules that act as potent regulators of gene expression in eukaryotic and prokaryotic organisms. These non-coding RNAs play crucial roles in cell development, growth, differentiation, proliferation, death and chromosome structure (19). Furthermore, it has been revealed that there is altered expression of mi-RNA genes in many human malignancies and cancers (20). Many gene instabilities have also been shown to be correlated to the production of mi-RNAs, and a hypothesis for their roles in the progression of tumors continues to be developed (21). Moreover, it has been established that mi-RNAs regulate a great number genes including genes involved in tumor etiology, like BRCA1, at the translational level (22).

The biogenesis of mi-RNAs is initiated from the nucleus as transcripts from non-coding DNA sequences. Drosha, a RNase III endonuclease, cleaves pri-mi-RNA in the nucleus and converts it into pre-mi-RNAs (22). The Exportin 5/Ran GTPase pathway leads to export of

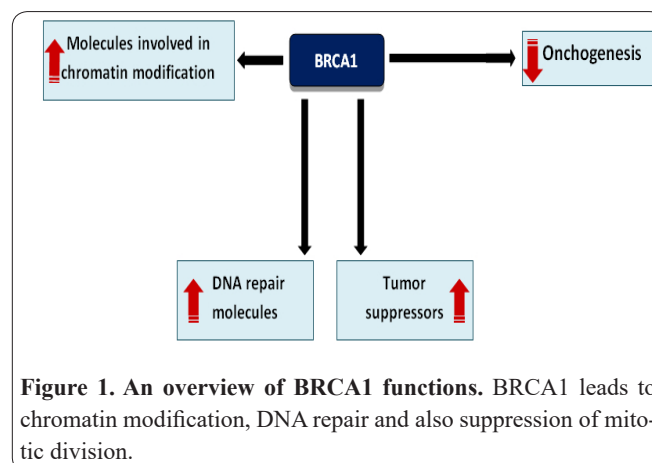
Pre-mi-RNAs into the cytoplasm where Dicer, a cytoplasmic RNase III endonuclease, cleaves the molecule and produces a short dsRNA duplex (22). The antisense strand of the duplex is directed into the RNA-induced silencing complex (RISC) which associates with complementary sequences typically at the 3'UTR of mRNAs, leading to translational silencing and/or RNA cleavage (23).

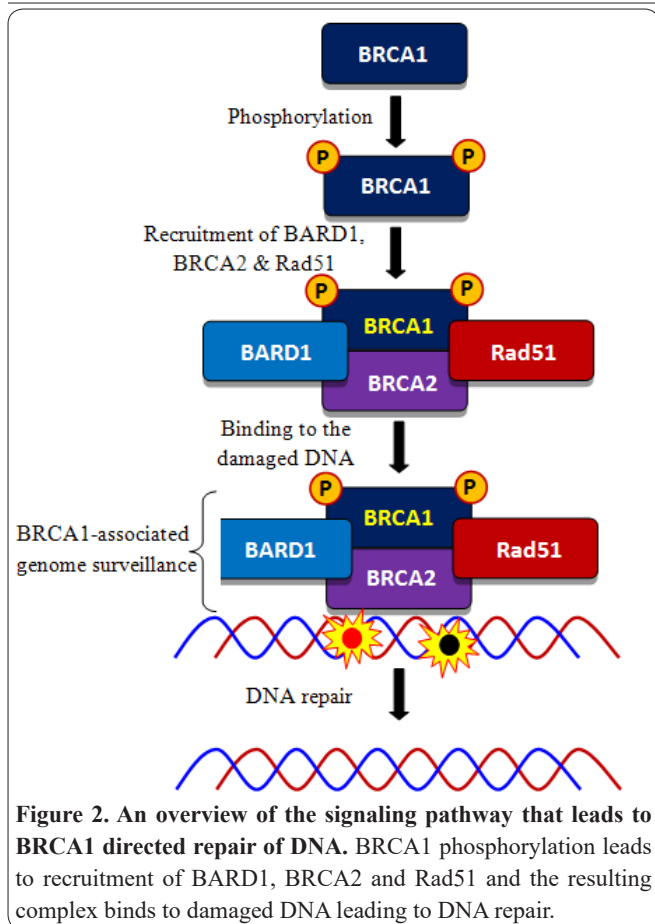
More than 1000 mi-RNA genes were found in the human genome and researchers believe that specific mi-RNA signatures can be correlated with both normal or cancer cell types (23). Additionally, recent data regarding the target genes of mi-RNAs has identified several mi-RNAs, that target immune related and cell replication regulating genes and now there is growing interest in identifying mi-RNA target genes. The following section describes recent studies regarding the main mi-RNAs that participate in the regulation of expression of BRCA1 in OC patients.

BRCA1

BRCA1 is a tumor suppressor gene located at 17q2, (41,196,312 to 41,277,500) and is expressed in all human cells. The Breast cancer type 1 susceptibility protein plays a role in DNA repair, DNA replication, chromosomal stability, control of cell cycle checkpoint, regulation of transcription, apoptosis, and unfolding of chromatin. BRCA1 induces expression of tumor suppressors and DNA repair proteins in a positive feedback manner and suppresses expression of oncogenes as well (Figure 1) (24). Several of the known mutations within BRCA1 leads to inactivation of its related protein and hence, DNA repair is disrupted and, thus, the risk for cancers, including OC, is increased. The BRCA1 protein is unable to repair damaged DNA alone and needs several other proteins, including signal transducers, other tumor suppressors and DNA damage sensors (including BARD1, Rad51 and BRCA2) to make a complex called the BRCA1-associated genome surveillance (Figure 2) (25).

BRCA1 contains CpG islands within its promoter region, hence, alteration of its methylation leads to changes in its expression which is associated with several human cancers including OC (26-28). Interestingly, it has been documented that DNA methylation also alters expression of several other genes involved in cancer etiology such as E-cadherin, estrogen receptor and MGMT (26). Accordingly, we have reviewed the recent





investigations regarding the status of cytosine methylation within the BRCA1 promoter region derived from patients suffering from OC.

Ovarian cancer

OC is a malignancy which is associated with high morbidity because of late detection (29). Therefore, it is important to understand its epidemiology, detection, etiology, pathogenesis and symptoms.

Epidemiology of OC

Epidemiologic studies report that the most common gynecologic cancer is OC and it is the fifth highest cause of cancer related deaths in women (30). Additionally, OC is the most common neoplasm in the developed countries and it is estimated that there are 192,000 new occurrences of the disease per year (31). The incidence rate of OC is the 8th most frequent occurring cancer in Iranian women and it is estimated that 61 percent of Iranian OC patients will only survive for five years (32).

Detection of ovarian cancer

Transvaginal sonography (TVS), which is a noninvasive technique, and evaluation of serum biomarkers are the best tools to detect early-stage OC (33). TVS only detects tumors which lead to a significant increase in ovarian volume, hence, this is not a reliable tool for the detection of serous-type tumors which give rise to rapid metastases to other pelvic sites before ovarian enlargement (33). Accordingly, it appears that serum biomarker testing is more reliable than TVS to detect OC. Serum biomarker testing is a suitable screen for detection of OC because it is cost effective, easily administered, has minimal invasiveness, and is non-subjective

(34). CA-125 is the most well-known biomarker for OC (35). CA-125 is an altered transmembrane glycoprotein with a high molecular weight and is expressed in several tissues including the fallopian tube, endometrium, and endocervix (35). However, healthy ovarian epithelium does not express CA-125. Serum levels of CA-125 are increased by 50% and 90% in early-stage and advanced-stage OC patients, respectively (35). Human epididymis protein 4 (HE4) is another tumor biomarkers which is used for detection of OC (36). Interestingly, HE4 is more sensitive and specific than CA-125 to distinguish early-stage of OC (36). Despite the existence of these biomarkers, the mortality of OC is high because most OC are detected at a late stage.

Etiology of ovarian cancer

There are several risk factors which increase the chance of OC developing. They are classified as follows:

Family history

Previous studies demonstrated that, although gene mutations are not found in most women who develop OC, women with a family history of OC and breast cancer, are more at risk of developing OC in comparison to other women (37). Mutations in BRCA1 and BRCA2 significantly increase the risk of OC by 35% to 70% and 10% to 30%, respectively (37). Additionally, women with close relatives who suffered from other cancers are also at more risk of OC (37). Investigations have also identified that over 80 genomic regions can increase the risks of developing OC (38).

Age

Age has a direct relation with occurrence of OC and it has been demonstrated that the incidence of OC increases in women that are more than 65 years old. Furthermore, the disease has a higher incidence in post-menopausal women compared to pre-menopausal women (39).

Infertility

Previous studies revealed that pregnancy engages an immunoregulatory mechanism which can improve immune responses (40). Thus, it is plausible that women that have never been pregnant have a higher risk of developing OC when compared with women with a history of pregnancy. Previous studies have also confirmed the hypothesis and shown that women with a history of pregnancy had a lower risk of acquiring OC (41).

Early age of menstruation

Women who begin menstruation at an early age are more at risk of developing OC (42).

Endometriosis

Endometriosis increases the risk of OC developing by 30% (43). Interestingly, Danazol is a medication which is used for the treatment of endometriosis and has an increased link to OC risk (44).

Other factors

In addition to the factors mentioned above, other factors including foods, hormone therapy, and obesity can

increase the risk of developing OC (45, 46).

Pathogenesis of ovarian cancer

According to morphologic and molecular genetic studies a dualistic model which categorizes different types of OC has been proposed and divides the disease into two groups; type I and type II. Type I tumors are usually associated with low stages which includes clear cell, mucinous carcinomas, low-grade serous and endometriosis (47). In contrast with type I, type II tumors are associated with papillary, glandular and solid patterns. This type is very aggressive and exists in advanced stages (47). Two types of tumors are genetically different and accordingly, type I displays specific mutations in the various histologic cell types (47).

Symptoms of ovarian cancer

OC has vague symptoms in the early stages, hence, it is not easy to recognize at this stage. In fact, doctors previously thought that ovarian cancer had no symptoms (unfortunately, many still do). The symptoms of OC at early stages are similar to other diseases including temporary bladder problems, pre-menstrual and irritable bowel syndromes and OC can be diagnosed as differences in the persistence and/or the gradual decay of symptoms (48). In the progressive stage, ovarian cancer spreads to various parts of the body and results in a variety of symptoms including ascites anorexia, vomiting, nausea, weight loss, fatigue, constipation and gastrointestinal obstruction. Intestinal obstruction and decreased movement of the bowel content lead to severe abdominal pain. Ovarian cancer which developed to the liver results in increasing size of the liver and by induction of pressure on the diaphragm, leading to symptoms of pain and shortness of breath. The progressive stage can lead to bone and brain metastases which, in turn, can lead to various symptoms such as headache, seizures and muscle weakness (49, 50).

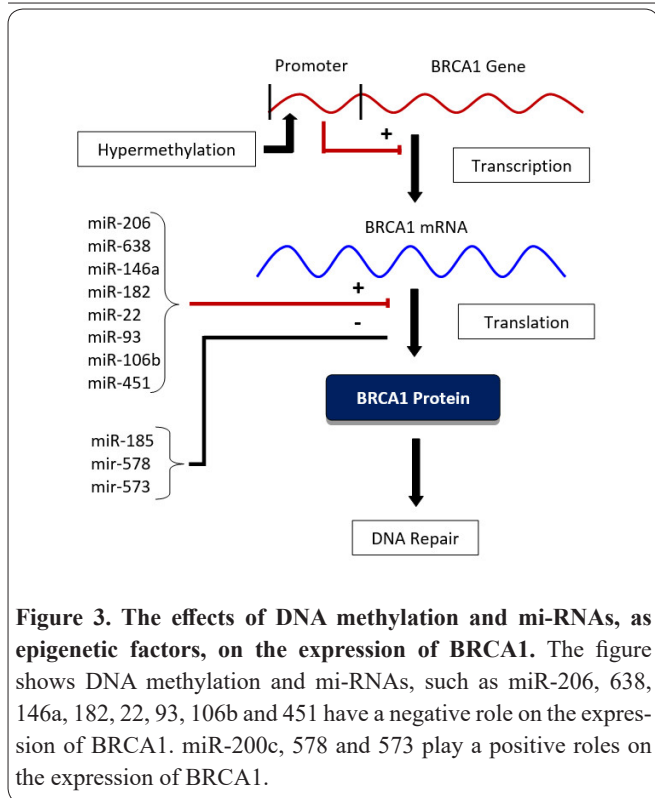
Methylation in the promoter region of BCRA1 in OC

Methylation within the promoter region of BCRA1

gene is speculated to play key roles in the initiation of cancer. Several studies have evaluated the status of BCRA1 gene methylation in OC patients within various ethnic and genetic backgrounds (table 1). Interestingly, most of the studies revealed that the promoter region of the BCRA1 gene is hypermethylated in OC tissues. Fewer studies reported that hypermethylation in the promoter region of BCRA1 was less than 10 percent. For instance, Geisler and colleagues or Esteller and colleagues evaluated the BCRA1 promoter methylation status for more than 200 OC samples and reported that hypermethylation was seen in less than 10 percent of samples (51). Most of the reported investigations revealed that the rates of hypermethylation in BCRA1 promoter region are between 10 to 20 percent. Reports from studies by Baldwin *et al.*, Catteau and colleagues, Ruscito *et al.*, Cunningham *et al.*, which were performed on various populations with different sample sizes (12 to 482) showed that the rate of hypermethylated cases were between 10 to 20 percent (52-65). According to the reported data it appears that, although some patients had hypermethylation lower than 10 percent and some are more than 40 percent, the most common BCRA1 promoter hypermethylation rates were between 10 to 20 percent in OC patients. The data suggests that hypermethylation in the promoter region of BCRA1 may play significant roles in the induction of malignancy in ovarian tissue by down-regulation of BCRA1 (Figure 3). However, as shown in table 1, the majority of patients had no hypermethylation in the promoter region of BCRA1, hence, it is plausible that other epigenetic factors participate in the regulation of BCRA1 expression during the transformation of normal ovarian cells. Accordingly, our previous study demonstrated that the methylation status of the promoter region of the BCRA1 gene in malignant tissues of Iranian OC patients were not significantly different with healthy tissues (8). One plausible explanation could be caused by the regulation of BCRA1 expression by miRNA. There are several studies which suggest an important role is played by miRNAs during the induction of malignancies. The following section describes the important mi-RNAs which

Table 1. The methylation status of the promoter region of the BCRA1 gene in ovarian cancer samples.

Authors	Number of ovarian cancer samples	Number (percent) of BCRA1 promoter hypermethylated	References
Nikbakht <i>et al</i> 2012	60	8 (13.33%)	(Nikbakht <i>et al.</i> , 2012)
Baldwin <i>et al</i> 2000	98	12 (12.24%)	(Baldwin <i>et al.</i> , 2000)
Catteau <i>et al</i> 1999	43	4 (9.3%)	(Catteau <i>et al.</i> , 1999)
Ruscito <i>et al</i> 2014	257	38 (14.78%)	(Ruscito <i>et al.</i> , 2014)
Cunningham <i>et al</i> 2014	482	52 (10.78%)	(Cunningham <i>et al.</i> , 2014)
Dobrovic <i>et al</i> 2014	154	20 (12.98%)	(Dobrovic <i>et al.</i> , 2014)
Wiley <i>et al</i> 2006	234	45 (19.23%)	(Wiley <i>et al.</i> , 2006)
Chiang <i>et al</i> 2006	63	11 (17.46%)	(Chiang <i>et al.</i> , 2006)
Rathi <i>et al</i> 2002	49	5 (10.2%)	(Rathi <i>et al.</i> , 2002)
Lambie <i>et al</i> 2003	20	2 (10%)	(Lambie <i>et al.</i> , 2003)
Yang <i>et al</i> 2011	316	33 (10.44%)	(Yang <i>et al.</i> , 2011)
Hilton <i>et al</i> 2002	92	12 (13.04%)	(Hilton <i>et al.</i> , 2002)
Wilcox <i>et al</i> 2005	50	8 (16%)	(Wilcox <i>et al.</i> , 2005)
Chan <i>et al</i> 2002	30	15 (50%)	(Chan <i>et al.</i> , 2002)
Alvarez <i>et al</i> 2005	34	15 (44.11%)	(Alvarez <i>et al.</i> , 2005)



have been evaluated in OC patients.

Commonly expressed Micro-RNAs in ovarian cancer and their effects on the translation of BRCA1

Genomic instability is considered one of the main causes of cancers and is also correlated to the expression pattern of mi-RNAs and there appears that there is a relationship between mi-RNA production and BRCA1 expression which may promote the development of OC. Expression levels of BRCA1 are frequently disrupted in OC patients, and it may be hypothesized that some mi-RNAs play key roles in the observed decreased expression of BRCA1 in some OC. It has recently been shown that elevated levels of miR-22, miR-93, miR-106b, miR-451 were detected in the serum of OC patients (66). Moskwa *et al.*, demonstrated that BRCA1 translation can be regulated by the Argonaute 1 (AGO1)/miR-182 complex and that BRCA1 transcripts are selectively associated with the complex (67). Moreover, they demonstrated that miR-182 overexpression was associated with the down-regulation of BRCA1 (67). Furthermore, treatment with a miR-182 antagonist leads to the up-regulation of BRCA1 (67). In addition, it has been documented that miR-146a can bind to the 3'-UTR of BRCA1 mRNAs and regulates its expression (68). The negative roles played by miR-638 (69) and miR-206 (70) on the expression of BRCA1 have also been demonstrated. In contrast, Erturk *et al.*, showed that BRCA1 mutations cause decreased expression of miR-200c (71). Thus, it seems that this mi-RNA is produced only in the presence of wild-type BRCA1. Danza *et al.*, revealed that expression levels of miR-578 and miR-573 were decreased in BRCA1/2-related breast cancer (72). A study by Tang and colleagues demonstrated that miR-185 up-regulates expression of BRCA1 (73). So, it appears that miR-578, miR-573 and miR-185 may participate in regulating expression levels of BRCA1 in a positive manner (Figure 3). It has been reported that

binding of mi-RNAs to the 5' UTR region of RNAs can lead to up-regulation of the target by providing a suitable scaffold for binding of essential translation factors (74, 75). Mi-RNAs also mediate up-regulation of some mRNAs by binding to the 5'UTR of the mRNAs which results in accelerating target mRNA interaction with polysomes (76, 77). The molecules also up-regulate translation through competition with decay pathways and expression inhibitors (78). Therefore, it seems that a balance between positive and negative mi-RNAs is important for the appropriate expression of BRCA1.

Conclusion

According to the data presented here, it may be concluded that expression of BRCA1, a protein known to be involved in transformation of ovarian cells, may be regulated by epigenetic factors including methylation of its promoter region along with several mi-RNAs. Accordingly, the up-regulation of BRCA1 can be considered as a potential target for the treatment of OC, provided the diseased tissues still carries wild-type copies of the gene. Hence, future therapies may be directed towards modulation or hypomethylation in the promoter region of BRCA1. Alternatively, novel molecular therapies that down-regulate the expression of mi-RNAs which negatively control BRCA1 translation could be considered. Additionally, it has been shown that miRNAs can directly modulate the methylation of genes, hence, one approach that could be considered is the use of specific miRNAs to regulate expression of BRCA1 through both the demethylation of its promoter and the upregulation of BRCA1 translation in OC. Furthermore, a more indirect approach could be considered which targets the regulation of DNA methyltransferases. Although several options can be considered, a great deal of work still needs to be completely before the regulatory pathways of BRCA1 can be fully characterized and can be considered for translational research in the clinic.

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