Molecular epigenetics in the management of ovarian cancer: are we investigating a rational clinical promise?

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INTRODUCTION TO EPIGENETIC MODIFICATIONS

Although genetic alterations, such as gene copy-number variations, contribute to the development of cancer, classical genetics alone does not account for all acquired characteristics of cancer cells. For this reason, it is generally appreciated that epigenetic abnormalities are involved in tumorogenesis. The definition of epigenetics is the potentially permanent and heritable change in gene expression, which is not attributed to any alteration in the underlying DNA sequence, but results from structural adaptations and responsive outcomes on chromosome regions (1, 2). Epigenetic modifications among cancer cells result in aberrant gene expression via DNA methylation, histone modifications, and non-coding microRNAs (miRNAs) and can also include alterations among transcription factors (3), although the latter is less often emphasized in epigenetics. These modifications are associated with initiation and progression of ovarian cancers (Figure 1).

DNA methylation is the most frequently studied epigenetic phenomenon. DNA methylation occurs among cytosine residues in cytosine–guanine (CpG) dinucleotides, which are mostly distributed in the CpG-rich regions referred to as “CpG islands” (4). This type of methylation is achieved by DNA methyltransferases (DNMTs), which are a family of enzymes that serve to transfer methyl groups onto DNA (5). In humans, DNMTs are divided into two groups: DNMT1 and DNMT3.

Changes in DNA methylation regulating gene expression are widespread, appearing in both normal and cancerous cells. For example, roughly 80% of CpG dinucleotides in the human genome are subject to methylation changes throughout life. In addition, nearly 70% of all CpG islands are methylated at any given time (6). Furthermore, in normal cells, DNA methylation regulates the silenced allele of imprinted genes and also represses expression of potentially harmful DNA transposon sequences (7). Interestingly, alterations and deregulation of epigenetic events precede the transformation that generates cancer cells (8).

EPIGENETIC MODIFICATIONS IN CANCER

Among cancer cells, DNA hyper-methylation is associated with gene silencing and DNA hypo-methylation with gene expression, both of which are widespread characteristics of malignancy (Figure 2). Most often, hypermethylated CpG islands within the DNA silence critical tumor suppressor genes, wrecking havoc on the cell’s ability to repair DNA damage, control cell growth, and inhibit proliferation. On the other hand, DNA hypo-methylation contributes to oncogenesis when previously silenced oncogenes become transcriptionally activated. In addition, DNA hypo-methylation can activate latent transposons and cause chromosomal instability in specific pericentromeric satellite regions (9–14).

Histone modifications also play important roles in epigenetic regulation. Histones are dynamic proteins that can become methylated or acetylated on specific amino acid residues, which correlates with active or repressive transcription (15, 16). An octamer
of histones make up the nucleosome, which is the fundamental building-block unit of chromatin. The nucleosome contains lysine-rich histone tails extending outward from the four constituent core histone proteins (H2A, H2B, H3, and H4). These histone tails provide sites for reversible modifications to alter chromatin structure and thus, gene expression. By tightly winding and condensing chromatin or loosening up the structure of chromatin, transcription factors and other proteins are prevented or permitted access to the DNA for transcription, respectively. The target residues of histone modifications are lysine (acetylation, methylation, and ubiquitination), arginine (methylation), and serine and threonine (phosphorylation). The crosstalk between histone modifications is complicated and varied based on chromosomal domains.

Overall, the combination of histone modifications contributes largely to chromatin pattern and gene expression (17). In general, histone acetylation adds more negative charges to positive lysine, thus loosening the electrostatic interaction between histones and the DNA backbone. For this reason, the condensation of chromatin is partially regulated by histone deacetylases (HDAC), a class of deacetylating enzymes that remove acetyl groups from lysine residues of histones, ultimately causing the repression of gene expression (18, 19). If methylation also targets the same lysine residue, which means excluding acetylation, the histone methylation will have the opposite effect, compared to acetylation, and repress gene expression. However, it is not an all-encompassing rule for every single case. In fact, the situation is much more intriguing. Indeed, methylation can
As previously stated, DNA methylation can prevent the transcription of genes involved in the regulation of the cell cycle and the promotion of apoptosis, which are important to maintain homeostasis. (21). Other types of histone modifications, such as ubiquitination and SUMOylation, are not discussed in this review. To further convolute this process, evidence suggests that regulating gene expression may occur through crosstalk between histone modifications and DNA methylations. (22–24).

MicroRNAs are small, non-coding RNAs, which are about 18–25 nucleotides in length. They negatively regulate gene expression through complementary binding to the 3′ UTR region in the promoter of targeted mRNAs, leading to mRNA degradation or translational repression, which is dependent on the level of complementarities. (25, 26). Because of their unique functions, miRNAs regulate many biological changes and contribute to cancer progression. For instance, studies comparing miRNA profiles between normal and cancerous specimens identified alterations of multiple miRNA during cancer development and progression. (27, 28). MicroRNAs can have dual roles in cancer progression, as tumor suppressors that repress oncogenes or as tumorigenesis factors that deregulate tumor suppressor genes. (29).

EPIGENETIC CHANGES IN OVARIAN CANCER

Role of DNA methylation in the development of malignancy

As previously stated, DNA methylation can prevent the transcription of tumor suppressor genes. Examples of this occurrence in ovarian cancer include the human Mutl. homolog 1 (hMLH1) and breast cancer susceptibility gene 1 (BRCA1), which are two critical genes that transcribe proteins involved in the DNA damage response and DNA mismatch repair. These processes are critical in maintaining a stable chromosome and fixation. DNA damage in cancer, the promoter regions of genes encoding these two proteins are hypermethylated, leading to the low expression levels of hMLH1 and BRCA1. Indeed, women with genetic mutations in BRCA1/2 are susceptible to breast, ovarian, and sometimes pancreatic cancer due to this aberration. Among older women with ovarian cancer, tumors are hypermethylated, leading to suppression in transforming growth factor (TGF)-beta pathway activity. (33). Other silenced genes in ovarian cancer include Ras-association domain gene family 1A (RASSF1a), lost on transformation 1 (LOT1), death-associated protein kinase (DAPK), target of methylation-induced silencing (TMS1)/apoptosis speck-like protein containing a CARD (ASC) (34–37), and insulin-like growth factor binding protein (IGFBP-3) (38). These genes encode proteins involved in regulation of the cell cycle and the promotion of apoptosis, which are important to maintain homeostasis.

Role of histone modifications in malignant tumorigenesis

Carcinogenesis and tumorigenesis are multifaceted; how normal tissue precisely undergoes stepwise changes to yield ovarian cancer, and then that progresses unregulated by mechanistic controls is largely debated. However, many aspects involved in the progression of ovarian malignancy are reported, including the role of histones in this process. For example, normal epithelial ovarian cells repress the expression of claudin-3 and claudin-4, yet these proteins are highly overexpressed in ovarian cancer. For claudin-3, this change is exclusively attributed to repressive histone marks, whereas claudin-4 repression occurs through histone modifications and DNA methylation. (39). These findings explain the molecular mechanisms of repressive histone marks likely occurring during tumorigenesis; the rationale is that claudins are critical components of tight junctions and other claudin family members comprise gene signatures leading to worsened outcomes in ovarian cancer. (40).

Another example of histone modifications affecting cell–cell interactions occurs in the TGF-beta1 receptor. This receptor is an important regulator of cell growth, cell cycle, and it also activates SMAD transcription factors. Interestingly, aberrant signaling of TGF-beta1 receptor results in histone modifications and repressive chromatin in ovarian cancer, which prevents the expression of ADAM19, the protein containing A Disintegrin and A Metallo-protease (41). ADAM19 is a metalloproteinase involved in cell–cell interactions and cell adhesion. Taken together, these studies suggest that histone modifications may be important epigenetic events allowing cells to alter contact with their environment.

MicroRNA alterations in the malignant progression of ovarian cancer

In ovarian cancer, miRNAs play a role in malignant progression. Evidence of this comes from reports that 37.1% of the miRNA genomic loci exhibit alterations in DNA copy-number. (42). Other molecular mechanisms of miRNA deregulation include DNA methylation and histone modification of miRNA genes. (43). Many profiling studies performed in ovarian cancer models illuminate abundant alterations. The results show that the miR-200 family and let-7 family are aberrantly regulated (Table 1) along with deregulated tumor suppressor miRNAs: miR-15a, miR-34a, and miR-34b. (43, 44).

In addition to genetically related explanations for miRNA deregulation, there are also changes in regulatory proteins that affect miRNA processing machinery. For example, there is a
reduction in the protein expression levels of Dicer and Drosha. These two proteins are essential factors involved in the biogenesis of miRNAs (49). A loss of one dicer allele facilitates tumorigenesis while a loss of both alleles in lethal to the cell (50). Furthermore, low expression levels of Dicer and Drosha correlates with poor clinical outcomes (49).

Significant interplay is likely between miRNAs and other molecular epigenetic mechanisms of DNA methylation and histone modifications. For instance, let-7a-3 is hypermethylated in ovarian tumor samples, and the suppression of this miRNA correlates with good prognosis (51). As another example, the down-regulation of miR-101 will de-repress its target EZH2, which is a catalytic subunit of the polycomb repression complex 2 (PRC2). Because the complex tri-methylates histone H3 lysine 27, its restoration aberrantly silences multiple tumor suppression genes in cancer (45, 52, 53). MiR-140, which targets histone deacetylase 4 (HDAC4), is also reported to be down-regulated in ovarian cancer (45, 54).

EMERGING POTENTIAL OF EPIGENETICS IN THE DIAGNOSIS OR PROGNOSIS OF OVARIAN CANCER

DNA METHYLATION TECHNIQUES IN OVARIAN CANCER DIAGNOSTICS AND PROGNOSTICS

Analysis of DNA methylation status among tumor specimens is the most favored approach for developing a biomarker diagnostic/prognostic due to methylation stability, amplification ability, high-sensitivity, and relatively low cost. In fact, DNA methylation has demonstrated diagnostic and prognostic use in other types of cancers, in particular glioma (55). To detect DNA methylation, the simplest approach is to treat cells with DNA methyltransferase inhibitors (DNMTIs). The treatment will reverse the DNA methylation and result in re-expression of genes that were silenced by this mechanism. Comparison of mRNA expression levels before and after treatment will suggest candidates of methylation in cancer, which can be confirmed using additional methods. An alternative approach uses HpaII, a methylation-sensitive restriction enzyme to digest genomic DNA samples prior to the amplification of digested DNA (using PCR) to compare differences in methylation levels between samples (56). In a more straightforward way, another method uses an antibody against 5-methylcytosine to precipitate methylated DNA fragments (DNA immunoprecipitation or MetDIP) (57). Collected genomic DNA fragments are then identified with array-based comparative genome hybridization to reveal human methylome maps (58).

Despite their readiness, array-based DNA methylation analysis approaches provide limited information about the extent and pattern of methylation in specific CpG regions. To overcome this drawback, bisulfite sequencing methods have been developed. Bisulfite treatment converts unmethylated cytosine residues to uracil, while methylated cytosine residues stay intact. After treatment, specific primers are used in methylation-specific PCR to amplify and help differentiate unmethylated and methylated DNA regions (59). This method is used to identify the difference in DNA methylation profiles in three major types of gynecological cancers: ovarian, endometrial, and cervical cancers (60).

To date, multiple other techniques applying bisulfite treatment have been introduced for whole-genome methylation sequencing and profiling. These include bisulfite padlock probes (BSPP), solution hybrid selection bisulfite sequencing (SHBS-seq), array capture bisulfite sequencing (ACBS-seq), and bisulfite-patch PCR (61–66); comprehensive information on methylation profiling is reviewed in Ref. (63, 67). In ovarian cancer, MethylCap-Seq for whole-genome DNA methylation profiling is a method using specific protein to capture methyl-CpG followed by high-throughput sequencing. MethylCap-Seq has been applied to analyze methylocnic patterns of ovarian tumors and results suggest that hedgehog signaling pathway members (ZIC1 and ZIC4) are DNA methylation prognostic biomarkers for ovarian cancer (68, 69).

miRNA – A BIOMARKER FOR OVARIAN CANCER?

Regarding the diagnosis of ovarian cancer among unsuspecting patients, there is no early detection biomarker that is used during routine gynecological examinations of otherwise “healthy” individuals. Although there are many candidates and extensive ongoing research for biomarkers of early stage ovarian cancer, biomarkers like CA-125 and CEA are limited to management of confirmed cases. Since it is always desirable to detect malignancy in the early stages with a minimally invasive method to collect samples, the bar is set very high for this endeavor. In addition, the accuracy requirement of a biomarker in a rare malignancy like ovarian cancer is exceedingly challenging.
In ovarian cancer, it is hypothesized that the detection of miRNAs present in circulation may be able to meet this challenge. The rationale for miRNAs as favored candidate biomarkers is due to the simplicity of obtaining blood samples and high-sensitivity detection methods. In addition, miRNAs are found in circulation within protected exosomes, which are small vesicles released into the extracellular environment from many types of cells, including tumor cells (81, 82). Interestingly, the transfer of circulating miRNAs among cells is hypothesized to be a method for internal communication within the body, similar to hormones; thus, supporting the idea of a screening approach involving miRNAs (82, 83).

There are many examples from the literature supporting the concept of miRNA biomarkers for ovarian cancer. Studies show that the expression levels of eight miRNAs have prognostic value in ovarian cancer: miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205, and miR-214 (45). Another study identified the expression of 21 miRNAs significantly different between ovarian cancer and normal serum specimens, including three known oncogenic miRNAs (or “oncomirs”) with biomarker potential: miR-21, miR-92, and miR-93 (84). Additionally, a large study using 300 plasma samples from ovarian cancer patients and 200 healthy controls evaluated circulating miRNAs and concluded that these are stable and specific. In this study, miR-205 and let-7f were significantly reduced in cancer specimens compared to normal controls. Moreover, let-7f has a lower expression level, which correlates with poor prognosis (85). Finally, another study suggests that among tumors, miR-9 and miR-223 deregulation is a biomarker of recurrent ovarian cancer (86).

Since miRNAs are released by cells into exosomes, studies have examined the viability of using exosomal miRNA as a potential biomarker. In this regard, research successfully used anti-epithelial cell adhesion molecule (EpCAM) to isolate exosomes secreted from ovarian tumors in plasma and compared exosome-containing miRNA expression profiles between samples from cancer patients versus healthy controls (87). Taylor and Gercel-Taylor intriguingly demonstrated that the level of tumor-derived circulating exosomes is higher in cancer patients than in normal controls. Furthermore, the level of increase correlates with higher disease grade when the exosomes are presented as concentrated protein. Finally, this study also showed that miR-9 and miR-223 deregulation is a biomarker of recurrent ovarian cancer (88).

EPÍGENETIC THERAPY IN OVARIAN CANCER

EXPLOITING DNA METHYLATION FOR THERAPEUTIC MANAGEMENT

Approaches to exploit DNA methylation changes for ovarian cancer therapy

To reiterate, regions of the DNA experience changes in both hyper- and hypo-methylation during cancer initiation and/or progression. In ovarian cancer, data suggest a correlation between global and satellite DNA hypo-methylation with malignancy since an overall increase in hypo-methylation is observed among ovarian cancer tissues, in comparison with normal controls (89, 90). Furthermore, the phenomenon of satellite DNA hypo-methylation is an independent marker of poor prognosis (91).

Methylated genes are known in ovarian cancer and exhibit diagnostic potential. A study using methylation-specific PCR to screen ovarian tumor samples for six tumor suppressor genes (BRCA1, RASSF1A, APC, p14ARF, p16INK4a, and DAP-kinase) indicated that this “hyper-methylation panel” provides diagnostic information in ovarian cancer. In addition, this study further suggests that the panel is 82% sensitive and 100% specific for the detection of ovarian cancer using patient serum DNA in stage I (92).

The technology also holds potential use for ovarian cancer-specific prognostic information. For example, methylation-specific PCR analysis of tumor tissues from 270 patients identified that IGFBP-3 gene promoter hyper-methylation is associated with a higher risk of disease progression and mortality. Thus, hypermethylation of IGFBP-3 is hypothesized as a biomarker for ovarian cancer outcomes, especially for patients in early stages of the disease (93).

Due to the extensive aberrant DNA methylation in cancer and the reversible nature of these events, inhibition of DNMTs is a worthy therapeutic approach to re-express tumor suppressors. DNMTIs are categorized into nucleoside and non-nucleoside analogs based on their chemical structures and mechanisms of action. DNMT nucleoside inhibitors incorporate into DNA, trap and inactivate DNMTs in the form of a covalent-DNA adduct. On the other hand, non-nucleoside DNMTIs directly block DNMT activity without covalently trapping the enzyme, thus appearing to have less toxicity (94). 5,6-Dihydro-5-azacytidine (DHAC) is a nucleoside analog of DNMT1 and is in clinical trials for the treatment of ovarian cancer (95). Hydralazine, a vasodilator that treats hypertension, is also a non-nucleoside DNMTI in clinical trials for cervical cancer as both monotherapy and combination therapy (96, 97).

Pre-clinical studies and clinical trials exploiting DNA methylation for re-sensitization

Ovarian cancer is a treatment-intensive disease and clinics are most often re-populated by their own patients. First-line chemotherapy is initially very effective in the treatment of ovarian cancer patients, but the period of remission they achieve is often short-lived. Thus, many approaches seek to re-sensitize tumors to the previously effective drugs. In contrast, others hypothesize that because previous attempts to re-sensitize recurrent ovarian tumors to first-line therapeutics has failed, they suggest that initial combinations of compounds aimed at preventing chemoresistance is the best approach (98). However, since neither approach has achieved bona-fide, proof-of-principle, research into both approaches is ongoing.

Researchers are evaluating the application of DNA methylation for chemotherapy re-sensitization. For example, the hypermethylated of hMLH1 (human MutL homolog 1) inhibits the apoptotic response to platinum-DNA abduct formation from platinum chemotherapy. Thus, this hyper-methylation is considered a major molecular cause of acquired resistance to platinum chemotherapy in ovarian cancer (99). In addition, the presence of methylated hMLH1 DNA in plasma after chemotherapy predicts poor survival for ovarian cancer patients (100). Interestingly,
the hMLH1 activity is restored after treatment with 5-aza-2’-deoxycytidine (decitabine) and so is the re-sensitization of ovarian cancer to cisplatin (101).

Another example of DNA methylation and chemotherapy re-sensitization surrounds RAS-associated domain family protein 1a (RASSF1A). The promoter methylation of RASSF1A is highly associated with ovarian cancer (34). RASSF1A binds to tubulin and promotes microtubule polymerization and stabilization (102, 103). The presence of RASSF1A blocks genome instability induced by RAS (85, 104). RASSF1A also causes cell cycle arrest through blocking Cyclin D1 accumulation (105). For all these reasons, RASSF1A is an interesting target for restoration.

Many pre-clinical studies present evidence that DNMT inhibitors are efficient in de-repressing tumor suppressor genes. This intimates that DNMT inhibitors may have therapeutic potential in combination regimens to overcome resistance and/or provide synergistic effects (106, 107). For example, decitabine re-sensitizes chemoresistant ovarian tumor xenografts to cisplatin, carboplatin, temozolomide, and epirubicin (101). Restoration of RASSF1A by inhibiting DNMT also increases ovarian cancer cell sensitivity to paclitaxel (108).

Indeed, DNMT inhibitors are also showing some success in the clinic (Table 2). Decitabine is undergoing clinical trials with carboplatin for patients with recurrent, platinum-resistant ovarian cancer (109). A report of a phase II clinical trial of low-dose decitabine combined with carboplatin for heavily treated and platinum-resistant ovarian cancer patients showed positive results. Low-dose decitabine altered the methylation of genes in tumorigenesis pathways, including the demethylation of hMLH1, RASSF1A, HOXA10, and HOXA11, leading to re-sensitization to carboplatin, increased response rate, and prolonged progression-free survival (110).

Hypo-methylation treatment, on the other hand, due to its non-specific effects, can be detrimental. One known example is the Fanconi anemia (FANC)–BRCA pathway in ovarian cancer. The malfunction of genes in FANC pathway leads to devastating mutagen hypersensitivity (111). In cancer treatment, the FANC–BRCA pathway plays a critical role in the response of cells to DNA-crosslinking agents. However, it was observed that, in ovarian cancer, FANC is inactivated due to hyper-methylation, and the demethylation of FANC is associated with ovarian tumor progression and acquired cisplatin resistance (112). In addition, there are oncogenic genes overexpressed by hypo-methylation in ovarian cancer, such as synuclein-γ and mammalian heparanase (endo-beta-glucuronidase) (113–115).

### Table 2 | Epigenetic drugs in gynecological cancer trials.

| Drugs             | Other names | Group          | Types of diseases            |
|-------------------|-------------|----------------|-----------------------------|
| Valproic acid     |             | HDAC inhibitors| Cervical, ovarian cancers    |
| Belinostat        |             | HDAC inhibitors| Gynecological cancers       |
| Decitabine        |             | DNMT inhibitors| Ovarian cancer              |
| Hydralazine       |             | DNMT inhibitors| Cervical cancer             |
| Dihydro-5-azacytidine |       | DNMT inhibitors| Ovarian cancer              |

### HISTONE MODIFICATIONS: HISTONE DEACETYLASE INHIBITION IN CLINICAL TRIALS

Histone deacetylases are enzymes that remove acetyl groups and have long been studied for treatment of cancer, in general, as well as of gynecological cancers, specifically. Although HDAC overexpression occurs in many types of cancers (116, 117), siRNA silencing HDAC1 and HDAC2 inhibits growth and promotes apoptosis in ovarian and cervical cancer cells (118, 119). Similarly, HDAC6 facilitates oncogenic transformation in ovarian cancer (120). Thus, there is sufficient support for the rational targeting and inhibiting HDAC within the treatment of this malignancy.

Based on their chemical structures, HDAC inhibitors are divided into four majors groups: short-chain fatty acid, hydroxamic acid, cyclic tetrapeptide, and benzamide (121). For example, valproic acid, a reagent belonging to the short-chain fatty acid group (also known as an anti-epileptic and mood stabilizer) is in clinical trials for the treatment of cervical and ovarian cancers (122–124). Scriptaid, another HDAC inhibitor in the hydroxamic group, showed growth inhibition and apoptosis-inducing potential in ovarian and endometrial cancers (125). Apicidin, an HDAC inhibitor in the cyclic tetrapeptide group, is also studied for its anti-growth effects in ovarian and endometrial cancer cells (126).

The aberrant expression of HDACs in gynecological cancers is likely associated with de novo resistance and/or poor chemotherapeutic efficacy and thus, chemoresistance development. As with nearly all new drugs, HDAC inhibitors are proposed for combination therapy to strengthen therapeutic efficacy as well as to minimize chemoresistance. Valproic acid has been studied in combination with several cytotoxic drugs, such as methotrexate or epirubicin, for synergistic or antagonistic effects in other types of cancer (127, 128). Belinostat (PDX101), a novel HDAC inhibitor in the hydroxamic acid group, displayed anticancer effects as a single agent as well as in combination by increasing the acetylation of tubulin induced by docetaxel and the phosphorylation of H2AX induced by carboplatin (129). Belinostat is under phase II clinical trials for gynecological cancer treatment in combination with platinum or paclitaxel to enhance effectiveness and help overcome resistance (130–134). OSU-HDAC42 (or AR-42), another new short-chain fatty acid HDAC inhibitor, has anti-growth effects on ovarian cancer cells but not normal epithelial cells. The compound re-sensitizes platinum-resisted ovarian tumors in vivo to cisplatin and may have great potential for combinations with platinum agents (135).

### EXPLOITING miRNAs FOR RE-SENSITIZATION OF CHEMORESISTANT DISEASE

The goal of targeting miRNAs in cancer treatment is to down-regulate oncomirs, to inhibit miRNAs that will become oncogenic proteins, or to restore tumor suppressor miRNAs. Multiple techniques have been developed to target oncomirs, such as locked nucleic acid (LNA), miRNA sponges, miRNA masking, or small-molecule inhibitors (136–139). On the other hand, the most straightforward way to restore tumor suppressor miRNAs is to deliver pre-miRNA precursors or miRNA mimics. However, straightforward it may appear, it is the targeted delivery of these molecules that represents a major obstacle.
A critical clinical problem in ovarian cancer is chemoresistance. Multiple studies in the field have focused on the roles of miRNAs in overcoming resistance to chemotherapeutic agents. Many miRNAs are reported as expressed differently between chemosensitive and chemoresistant ovarian cell lines, such as miR-30c, miR-130a, and miR-335, among those, miR-130a is confirmed to target resistant factor M-CSF (Table 3) (140). In addition, the enforced expression of miR-30c-2-3p into chemoresistant and chemo-insensitive ovarian cancer cells significantly reduces their viability, independently of cisplatin or paclitaxel, without affecting immortalized ovarian surface epithelial cells (141).

Although the miR-200 family is a potential prognostic factor of ovarian and endometrial cancer (87, 155), it may have a role in re-sensitization. The low expression of miR-200c in cancer leads to an increase in the expression of its target, class III β-tubulin (TUBB3) (142). Since the expression of TUBB3 is required for chemoresistance to microtubule-binding agents (e.g., taxanes and vinca alkaloids), restoration of miR-200c downregulates TUBB3, and effectively re-sensitizes ovarian cancer cells to paclitaxel (142, 143, 156).

In addition to the miR-200 family, several members of the let-7 family are well documented as down-regulated in ovarian cancer, including let-7a, let-7b, let-7c, let-7d, and let-7i (44–48, 144). Among these, let-7a is a potential biomarker for the selection of chemotherapy in ovarian cancer. Patients with low let-7 showed good response using platinum-paclitaxel combination therapy, while patients with higher let-7a had better survival using platinum without paclitaxel; adding paclitaxel to this group produced worse progression-free and overall survival (147). The down-regulation of another member of the let-7 family, let-7i, is associated with resistance of ovarian cancer cells to cisplatin, which suggests that let-7i could be used as a therapeutic target to overcome platinum resistance and as a biomarker to predict chemotherapy response in ovarian cancer patients (144). Another study observed that the let-7 family member, let-7g, down regulates the multiple drug resistance 1 (MDR1) gene, one of the major factors causing paclitaxel resistance in ovarian cancer (145).

There are numerous other miRNAs that have roles in ovarian cancer chemoresistance with known mechanisms. These include, but are not limited to, miRNAs like miR-214, miR-27a, and miR-451. MiR-214 targets PTEN, a known tumor suppressor, therefore, inducing cell survival and cisplatin resistance (46). MiR-27a increases MDR1/P-glycoprotein expression in ovarian cancer cells by targeting HIPK2 as an intermediate (157). Similarly, miR-451 and miR-21 also facilitate MDR1/P-glycoprotein overexpression, leading to paclitaxel resistance in ovarian cancer cells (158, 159).

**TARGETING TRANSCRIPTION FACTORS IN OVARIAN CANCER**

Cancer is often a condition with aberrant gene expression, specifically involving the overexpression of oncoproteins. Altered transcription factors are recognized as an epigenetic entity comprising the “ovarian cancer cell epigenome” (3). This is not surprising given the relationship between transcription factors and structural (not sequence) alterations of the DNA (via DNA methylation and histone modifications).

There are numerous examples of aberrant transcription factors in cancer. Perhaps the most prominent of all is the tumor suppressor protein p53. Mutations of TP53, the gene encoding p53, are very common in ovarian cancer (160). In fact, nearly 100% of patients with high-grade serous epithelial ovarian cancer have

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**Table 3 | miRNAs involved in chemoresistance.**

| miRNAs                  | Trend in resistance | Target genes                          | Resisted drugs   | References |
|-------------------------|---------------------|---------------------------------------|-----------------|------------|
| miR-200 family          |                     |                                       |                 |            |
| Let-7i                  | Reduced             | B-tubulin III TGF-beta2, ZEB1         | Paclitaxel      | (142, 143) |
| miR-30c, miR-130a, miR-335 | Reduced            | M-CSF (target of miR-130a)           | Paclitaxel, cisplatin | (140) |
| miR-214                 | Increased           | PTEN                                  | Cisplatin       | (46)       |
| miR-27a, miR-21, miR-451 | Increased           | MDR1 (indirectly through HIPK2, in case of miR-27a) | Paclitaxel      |            |
| Let-7g                  | Reduced             | MDR1 (indirectly through IMP-1)       | Taxane agents   | (145)      |
| miR-27a, miR-23a, miR-30c, let-7g, miR-199a-3p, miR-378, miR-625 | Increased | Platinum agents                        |                 | (146)      |
| Let-7a                  | Decreased           | Caspase-3                             | Paclitaxel      | (147, 148) |
| miR-130b                | Increased           | KEAP1                                  | Cisplatin, paclitaxel | (149) |
| miR-141                 |                     |                                       |                 | (150)      |
| miR-106a, miR-591       | Increased (miR-106a) | BCL-10, caspase-7, ZEB1                | Paclitaxel      | (151)      |
| miR-29                  | Decreased           | COL1A1                                 | Cisplatin       | (152)      |
| miR-182                 | Increased           | PDCD4 TCEAL7                           | Paclitaxel      | (153, 154) |
mutations in p53. Overall, at least 50% of all ovarian tumors have mutations in p53, most of which are point mutations leading to amino acid substitutions. These are detrimental to the p53 protein because they affect the DNA-binding domain of the transcription factor (161). Unfortunately, therapeutic intervention using p53 as the target molecule has not yet achieved measurable success (98).

The transcription factor and tumor suppressor protein p53 are critical to the signaling pathways of cell cycle arrest and apoptosis. Once activated by DNA damage detection or UV radiation, p53 induces the expression of many well-known apoptosis inducers and other tumor suppressors, such as p21<sup>WAF1</sup>, BAX, PTEN, and TSP-1. Because of this important role, the inactivation of p53 facilitates many phases of tumor progression as DNA damage cannot be repaired and apoptotic pathways cannot be activated when necessary (161).

Beside p53, other transcription factors have important roles in ovarian cancer pathology. For example, Gill1 (glioma-associated oncogene homolog 1) expression is elevated in advanced serous ovarian cancer and this event is correlated with unfavorable survival (162). Since transcription factor alterations can have a tremendous impact on the balance of the entire biological system, targeting transcription factors is an emerging trend in cancer therapy research. The possibility of exerting broad control over the system could be a powerful method of regaining regulatory control. This is especially in light of lessons learned in other cancers whereby targeting one particular kinase or protein in a larger signaling pathway leads to the rapid acquisition of therapeutic resistance.

On the other hand, inhibiting particular transcription factors could provide specificity toward malignant overexpression events in cancer (e.g., oncogenes, oncomirs, etc.). Furthermore, this approach is appealing because it might produce more tolerance among healthy cells due to redundancies in normal signaling pathways. Two major approaches in targeting transcription factors are post-transcriptional silencing (using siRNAs or miRNAs) or blocking the binding of transcription factors to DNA during activation. Another indirect approach is regulating histone-modified enzymes and DNA methyltransferase if the target transcription factor is mis-regulated through histone modification and/or DNA methylation.

Many well-known transcription factors are studied as potential targets in general cancer treatment, such as STATs, NF-κB, and Notch1 (163–165). In gynecological cancers, multiple studies have reported the involvement of transcription factors in cancer progression and described them as potential targets for cancer treatment. In ovarian cancer, the blockage of STAT3 using a decoy oligodeoxynucleotide inhibits cancer cell growth (166). Another study in ovarian cancer also showed that suppression of NF-κB activity using minocycline, a tetracycline, had beneficial effects both in vitro and in vivo (167). More research is needed in this area to refine this approach and evaluate its worthiness.

**CONCLUSION: EPIGENETIC THERAPY**

By undertaking research projects focused on epigenetic-related translational applications, are basic scientists investigating a rational clinical promise? To address this question, it is necessary to review the successful progression of ideas from the laboratory into clinic therapeutics. Although no epigenetic drugs have advanced into the clinic for use against ovarian cancer, there are several FDA-approved therapeutics (e.g., vorinostat, decitabine, and romidepsin) for other types of cancer, especially liquid tumors. Clinical trials are ongoing for ovarian cancer with epigenetic therapeutics (Table 2). Since first-line therapy often results in disease remission, predictions support using new drugs in combination therapy. Although hope lingers for PARP inhibitors, this class of drugs may only treat a specific population of women (168). Whether using epigenetic modifiers will achieve significant improvements in overall survival is calculable. Nevertheless, to advance patient outcomes in ovarian cancer, new approaches are required — the previous breakthroughs occurred in 1978 (cisplatin) and 1992 (paclitaxel). An improved therapeutic regimen for ovarian cancer is long overdue. Epigenetics provide hope in a new direction.

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