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REVIEW

Epigenetic Therapies as a Promising Strategy for Overcoming Chemoresistance in Epithelial Ovarian Cancer

Dong Hoon Suh[1], Mi-Kyung Kim[2], Hee Seung Kim[2], Hyun Hoon Chung[2], Yong Sang Song[2,3,4]

[1]Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, [2]Department of Obstetrics and Gynecology, [3]Cancer Research Institute, Seoul National University College of Medicine, [4]Major in Biomodulation, World Class University, Seoul National University, Seoul, Korea

Over the past decades, prognosis of advanced stage epithelial ovarian cancer remains very poor, despite the development of new chemotherapeutic drugs, as well as molecular targeted agents. Late presentation and frequent chemoresistance account for the poor prognosis. Emerging studies have shown that many genetic changes, especially p53 mutation, are associated with the chemoresistance. However, recent failure of the clinical trials using p53 gene-therapy makes researchers discuss the possible reasons for the failure. Epigenetic changes are considered one of the substantial reasons. Successful restoration of the aberrant epigenetic changes may be a promising strategy for overcoming chemoresistance in epithelial ovarian cancer. Herein, we will summarize the rationale for epigenetic therapy of cancer and current status of epigenetic studies in relation to chemoresistance in epithelial ovarian cancer. **(J Cancer Prev 2013;18:227-234)**

Key Words: Epithelial ovarian cancer, Chemoresistance, Epigenetic therapies, P53

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer death in women in the US.[1] Since there is no effective screening tool and the lack of early presenting symptoms, patients are typically diagnosed late, when complete surgical cytoreduction is not possible. In addition to the late diagnosis, over 70% of high recurrence rate, especially for platinum-resistant tumor, keeps the 5-year survival rate for the patients with advanced epithelial ovarian cancer (EOC) below 30%.[1] Despite enormous efforts to develop effective anti-cancer drugs, there was little change in the poor outcome of EOC over the past decades. Therefore, the development of a novel therapeutic strategy is imperative for improving the survival of women with EOC.

P53 GENE THERAPY AS A DISAPPOINTING MASTER PLAN

1. P53 and ovarian cancer

A p53 is a master regulator of the apoptotic pathway and coordinates the programmed cell death at many levels through numerous mechanisms. Apoptotic pathways are considered to contribute to the cytotoxic action of several chemotherapeutic drugs including cisplatin, which is most commonly used in EOC. Loss or mutation of p53 has been reported to be causally associated with chemoresistance in EOC.[2-4] Mutation and loss of *TP53* function is one of the most frequent genetic abnormalities in ovarian carcinoma

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Correspondence to: Yong Sang Song
Department of Obstetrics and Gynecology, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea
Tel: +82-2-2072-2822, Fax: +82-2-762-3599, E-mail: yssong@snu.ac.kr

and is observed in 60-80% of both sporadic and familial cases.[5] Furthermore, *TP53* mutation and consequent overexpression are observed more frequently in advanced-stage than in early-stage and are associated with the poor survival.[6,7] It is possibly not only due to more aggressive phenotype but also due to the resistance to chemotherapy-induced apoptosis.[3,8,9]

The high frequency of *TP53* mutations in EOC and the central role of p53 in regulating apoptosis suggest that it would be an appealing target for gene replacement therapy.[10]

2. Failure of p53 gene therapy

Based on the promising results from preclinical and phase I trials,[11,12] a clinical trial of p53 gene-therapy was started for patients with primary stage III EOC with p53 mutations.[12] For the patients in the experimental group, 5-day p53 gene therapy was added to the standard therapy of carboplatin and paclitaxel from the 2nd to 6th cycle. Each cycle consisted of intraperitoneal administration of 10^{13} particles a day of replication-deficient wild-type p53 in a serotype-5 adenovirus.[10] However, the study was closed after the first interim analysis, which showed disappointing results. The addition of p53 gene therapy to standard treatment did not improve therapeutic effectiveness for patients with optimally debulked advanced EOC and actually increased treatment morbidity.[10] The plausible causes of the failure of the p53 gene-therapy study include inefficient vector system, neutralizing antiadenoviral antibodies in the ascitic fluid of patients, and the possibility of attenuation of the therapeutic effects of concomitantly administered cytotoxic drugs through the reactivated p53 pathways in tumor cells causing cell cycle arrest over cell death, and so forth.[13,14]

Despite the failure of trial, p53 is still a highly attractive druggable target for EOC given the central role of loss or mutation of p53 in chemoresistance of EOC. However, frequent epigenetic changes in tumor cells suggest that the presence of wild-type p53 gene in the genome of tumor cells might be no guarantee for its accurate expression and functionality.[10] So, the epigenetic changes have been extensively explored. Following the epigenetic studies, the development of effective epigenetic modulators has been done.

EPIGENETIC MODULATION AS A PROMISING THERAPEUTIC APPROACH

Epigenetic changes are defined as heritable changes in gene expression that occur without changes in the DNA sequence and include DNA methylation, histone modification, and posttranslational gene regulation by microRNAs (miRNAs).[15,16] DNA methylation is the best-studied epigenetic changes in the cancer cells. DNA methylation, the transfer of a methyl group to the carbon-5 position of cytosines, occurs almost always within the context of cytosine-guanine (CpG) dinucleotides in promoter of gene. In cancer, CpG islands can become hypermethylated, contributing for example to silencing of tumor suppressor gene like p53. The second intensively studied class of epigenetic mechanism is histone modification. Histones, the major components of chromatin, can undergo multiple post-translational modifications, such as acetylation and deacetylation by histone acetyltransferases (HATs) and deacetylases (HDACs), respectively. HDAC inhibitors (HDACIs) promotes accumulation of the acetylated form of histone proteins, leading to less-condensed packaging of genes in chromatin which may lead to the re-expression of silenced tumor suppressor genes. Compared with forementioned two epigenetic mechanisms, little is known about microRNAs (miRNAs). miRNAs are endogenous, small non-coding RNAs that negatively regulate gene expression. Numerous publications reported a crucial role of miRNAs in regulating gene expression and the association of differential expression of miRNAs in cancer cells with chemoresistance in EOC.[17,18] Though few miRNAs have so far been consistently found to be deregulated in many studies, consistent efforts are being made to elucidate the role of miRNAs in the development of chemoresistance in EOC.

In this review, we will mainly focus on DNA methylation and histone modifications in relation to chomoresistance in EOC.

1. P53-related epigenetic modulation and chemoresistance

Oshiro et al. demonstrated that mutation of p53 and

aberrant cytosine methylation of the specific gene promoter could cooperate in transcriptional repression in breast cancer cells.[19] Two p53-target genes, *MASPIN* and desmocollin (*DSC3*), are silenced in association with aberrant cytosine methylation of their promoters. While restoration of wild-type p53 alone can partially overcome the repressive barrier of DNA methylation, inhibition of DNA methylation with 5-aza-2'-deoxyazacytidine (decitabine) in combination with restoration of wild-type p53 status resulted in a synergistic reactivation of these genes to near-normal levels. This association of *MASPIN* regulation with p53 status and *MASPIN* promoter methylation was also found in EOC.[20] The combination of DNA methylation inhibitor (5'-azacytidine) and wild-type p53 transfection produced a 36% reduction in *MASPIN* promoter methylation and 4.5-fold increase in *MASPIN* transcription in SKOV3 cells, whereas the wild-type p53 transfection alone resulted in a 3.3-fold increase in *MASPIN* mRNA. These results suggest that epigenetic treatment may be a useful strategy to enhance the efficacy of gene therapy in EOC. However, there was a disparity in the results obtained in cell lines compared with clinical tumor specimens. In contrast to the result from in vitro experiments, *MASPIN* protein was 6 times more likely to be detected in p53 mutated EOC specimens relative to EOC specimens with a wild-type p53 gene. Further studies are necessary to explain the disparity.

There is also an evidence that supports the prominent role of epigenetic regulation of p53-related cell death mechanism in chemoresistance in ovarian cancer. Wolf et al.[21] reported the defective activity of a death signal downstream of the p53-gene, apoptotic protease-activating factor 1 (*APAF1*), in ovarian cancer cells resistant to p53-mediated apoptosis. Considering that methylation-inhibiting agents restored the expression of the *APAF1* gene, aberrant methylation in *APAF1* genes could compromise p53-mediated cell death by cytotoxic drugs.[22]

2. DNA methylation in ovarian cancer: driver versus bystander of chemoresistance

Various aberrant epigenetic alterations including DNA methylation are commonly observed in EOC (Table 1).[23]

Table 1. Epigenetic alterations and candidates for drivers of acquired chemoresistance in epithelial ovarian cancer

Event	Effect	Chromosome/type	Genes
DNA methylation			
Hypomethylation	Activation	NA	*IGF2, SNCG, MCJ, claudin-4, SAT2, BORIS*
Hypermethylation	Inactivation		
Candidates for drivers of acquired chemoresistance		Xq21.3-q22.2	*ARMCX2* (development, tissue integrity)
		7q32	*MEST* (development)
		3p22.3	*MLH1* (DNA mismatch repair)
Candidates for epigenetic biomarkers		NA	*BRCA1, RASSF1A, OPCML, SPARC, ANGPTL2, CTGF, ARHI, PEG3, DAPK1, LOT1, TMS1/ASC, PAR-4, CDH13, ICAM1, Hsulf-1, PALB2, TUBB3, HOXA9, HOXA10, HOXA11, PLAGL1, DNAJC15, MUC2, PCSK6, CDKN2A, CDKN1A, SOCS1, SOCS2, NTS, PYCARD, ARLTS1, DLEC1, PTEN, SFN, p16, COL1A1, ARHGDIB, FLNA, FLNC, GLUL, HSPA1A, MDK, NEFL, PSM B9*
miRNAs	Up-regulation	miR-200a	*BAP1, SIP1, ZEB1/2*
		miR-299-5p	*DLK1*
		miR-135b	*MSX2*
		miR-141	*BAP1*
		miR-200c, miR-200b	*BAP1, ZEB1/2, SIP1*
		miR-214	*PTEN*
		miR-302d, miR-373	*VEGFA*
	Down-regulation	miR-199a	*c-SRK, MMP13, FGF2*
		miR-140	*c-SRK, MMP13, FGF2, VEGFA*
		miR-145	*c-SRK, MMP13, FGF2, PARP8, IRS1*
		miR-15/16	*BCL2*
		miR-130b	*CSF-1*

Adapted from Despierre et al.[15] and Zeller et al.[24] and updated with the most recent literature findings. NA, not applicable.

There is growing evidence that epigenetic changes such as hypermethylation of CpG islands in promoter regions of tumor suppressor genes result in transcriptional silencing. The hypermethylation of CpG islands is likely associated with the acquisition of drug resistance in EOC.[24] However, only 5% of genes with hypermethylation at their promoter region showed the downregulated level of expression in cisplatin-resistant A2780/cp70 cell lines.[24] Moreover, treatment of A2780/cp70 with the demethylating agent (decitabine) induced resensitization to cisplatin and re-expression of only 17% of the downregulated genes. Thus, less than 1% of hypermethylated genes in platinum-resistant ovarian cancer cells might account for the acquisition of platinum resistance. That is, only a small proportion of hypermethylated genes might be epigenetic drivers related to the chemoresistance, whereas almost all hypermethylated loci in ovarian tumors are likely to be bystanders.[16,24] Zeller et al. identified consistent methylation and expression changes associated with chemoresistance.[24] *MLH1* was shown to have a direct role in conferring cisplatin sensitivity when reintroduced into cells in vitro and considered as a potential key driver of chemoresistance whose expression is silenced by DNA methylation. In addition, a higher degree of CpG island methylation was associated with early disease recurrence after chemotherapy.[25]

3. Histone modification in ovarian cancer

Histone modifications are epigenetic changes that are involved in chromatin structure regulating the access to the underlying DNA. The amino-terminal modifications of histone, such as methylation, acetylation and phosphorylation, dictate dynamic transitions between transcriptionally active or silent chromatin states. Thus, gene expression is in part determined by the pattern of associated histone modifications, known as the histone codes.[26] In addition, it is known that histone methylation can be linked to DNA methylation and consequently aberrant gene expression in cancer cells.[27] Several putative tumor suppressor genes including RASSF1, DLEC1, CDKN1A, CKN2A, and MLH1 are down-regulated by not only promoter methylation but also histone modifications (Table 1). Repressive histone trimethylation of lysine 27 on histone H3 (H3-K27me3) was shown to be responsible for RASSF1 down-regulation in EOC cells.[28] Removal of H3-K27 methylation resensitized drug resistant EOC cells to cisplatin by increasing access of the drug to target DNA sequences. This increased platinum-DNA access was likely due to the relaxation of condensed chromatin. On the other hand, Wei et al.[29] reported a significant lower level of H3-K27me3 in ovarian cancer tissue samples than normal ovarian tissue samples. They also showed that loss of H3-K27me3 might be a predictor of poor outcome in patients with EOC. EZH2, a histone methyltransferase, methylates histone H3 on lysine 27. Overexpression of EZH2 is known to contribute to acquire cisplatin resistance in ovarian cancer cells in vitro and *in vivo*.[30] More recently, Stronach et al.[31] showed HDAC4 mediated platinum resistance in ovarian cancer. Significantly enhanced apoptotic response to platinum treatment in resistant cells was observed following knockdown of HDAC4 or STAT1. STAT1 is activated in response to cisplatin treatment in acquired platinum resistant EOC cells. Interestingly, Acetyl-STAT1 was detected in platinum-sensitive cells but not in HDAC4 overexpressing platinum-resistant cells from the same patient. This suggests that HDAC4 interacts with STAT1, modulating its acetylation, thereby abrogating sensitivity to cisplatin. The result was confirmed by the fact that acquired platinum resistance was reversed by treatment with HDACIs, such as aroyl-pyrrolyl-hydroxy-amide-4a (APHA4a).

4. New findings from comprehensive epigenome study of the Cancer Genome Atlas

Specific patterns of methylation are known to occur for individual cancer type.[32] Recently, methylation and silencing in a high proportion of ovarian cancer cells were first reported by the Cancer Genome Atlas (TCGA).[33] The comprehensive microarray analyses of TCGA project produced high-resolution measurements of DNA promoter methylation for 489 high-grade serous ovarian cancer samples. A total of 168 genes were involved in increased promoter methylation events. Although DNA methylation was correlated with reduced gene expression across all samples, *AMT*, *CCL21* and *SPARCL1* were noteworthy because they showed promoter hypermethylation in the vast majority of the tumors.

TCGA showed that 20% of studied high-grade serous ovarian cancer samples had germline or somatic mutations in *BRCA1/2* and 11% lost *BRCA1* expression through DNA methylation. Epigenetic silencing of *BRCA1* was shown to be mutually exclusive of *BRCA1/2* mutations.[33] In this study, there was no significant difference of survival between patients with epigenetically silenced *BRCA1* and *BRCA1/2* wild-type tumors. However, overall survival of the patients with *BRCA1/2* wild-type tumors was worse than that of *BRCA1/2* mutated tumors. Mutually exclusive genomic and epigenomic *BRCA1* inactivation might explain different survival between the two groups.

5. Current status of epigenetic therapies in ovarian cancer

Based on the potential reversibility of various types of epigenetic changes, extensive preclinical and clinical studies have largely focused on two types of inhibitors: DNA methyltransferase inhibitors (DNMTIs) and histone deacetylase inhibitors (HDACIs) (Table 2).[34] DNMTIs and HDACIs induce re-expression of tumor suppressor genes through the release from epigenetic gene repression by preventing transfer of the methyl group and inhibition of deacetylation of histone proteins, respectively. Promising results of preclinical studies have been supported by the following clinical studies using DNMTI or HDACI for the resensitization of chemotherapy.[35-41]

Two phase I-II trials for patients with platinum-resistant or refractory ovarian cancer showed promising results that a hypomethylating agent may play a role in partially reversing platinum resistance for patients with ovarian cancer.[38,39] In a phase I trial by Fang et al., low-dose decitabine was administered daily for 5 days with two dose levels, 10 mg/m^2 for 7 patients and 20 mg/m^2 for 3 patients, before carboplatin at AUC 5 on day 8 of a 28-day cycle. One complete response and three stable diseases for ≥6 months were observed. The other study showed that sequential treatment with azacitidine and carboplatin for 30 patients (18 resistant and 12 refractory patients) resulted in 1 complete response, 3 partial responses, and 10 cases of stable disease. By contrast, conflicting result was offered by a clinical trial for patients with platinum-sensitive EOC.

A randomized phase II trial of the UK Cancer Research Group compared the combination of decitabine and carboplatin with single agent carboplatin for patients with platinum-sensitive recurrent ovarian cancer.[42] Decitabine was given at 90 mg/m^2 on day 1, with carboplatin at AUC 6 on day 8. Lower clinical response rate was observed for patients receiving the combination regimen than for those receiving carboplatin alone (0/11 versus 7/14 objective responses by RECIST criteria, respectively). There three clinical studies suggested that patients with platinum-resistant EOC might have some survival benefits from the addition of hypomethylating agents to the standard chemotherapy.

Recently, a growing number of literatures reported a synergistic effect of the combined treatments of DNMTI and HDACI in EOC cells. Steele et al.[43] showed that the combination of decitabine and HDACI (belinostat) resulted in a significant increase in the expression of epigenetically

Table 2. Clinical status of epigenetic modulators

Type of epigenetic modulators	Class of compound	Compound	Target	Development stage
DNA methylation inhibitor	Nucleoside analogue	5'-azacytidine [Vidaza]	DNMTs	Phase Ib-2a[38]
		5-aza-2'-deoxycytidine [Decitabine/Dacogen]	DNMTs	Phase I[39]
	Non-nucleoside analogue	Hydralazine	DNMT1	Phase II[45]
Histone deacetylase inhibitor	Hydroxamate	PXD101 [Belinostat]	Class I, II	Phase II[35]
		LBH589 [Panobinostat]	Class I, II	Phase II[46]
		Suberoylanilide hydroxamic acid [SAHA, Vorinostat]	Class I, II	Phase II[40]
		Trichostatin A	Class I, II	Preclinical
	Aliphatic acid	Valproic acid [VPA]	Class I, II	Phase II[46]
		Phenylbutyrate	Class I, II	Phase I[46]
	Cyclic tetrapeptide	Apicidin	Class I, III	Preclinical[47]
	Benzamide	MGCD0103	Class I	Phase II[46]

Adapted from Zeller et al.[34] with modification. DNMT, DNA methyltransferase; HDAC, histone deacetylase.

silenced MLH1 and MAGE-A1 in A2780/cp70 cell lines compared with decitabine alone. Furthermore, the combination markedly enhanced the effects of decitabine alone on the cisplatin sensitivity of xenografts. In addition to the expected synergistic effect of the combination treatment, Meng et al.[44] reported the superiority of DNA demethylation to histone acetylation for reactivating cancer-associated genes in EOC cells. Treatment with HDACI (trichostatin A) increased histone acetylation at the hypermethylated promoter, but with no demethylating effects and little effects on gene expression, *p16*, *hMLH1* and *MGMT*. However, decitabine caused DNA demethylation and increased histone acetylation at the hypermethylated promoter and resulted in reactivation of *p16*, *hMLH1* and *MGMT*. Combined treatments synergistically increased histone acetylation with the re-expression of the hypermethylated genes.

FUTURE PERSPECTIVES

p53 plays multiple roles as a master regulator with pleiotropic effects on metabolism, anti-oxidant defense, genomic stability, proliferation, senescence, an cell death[48]. Therefore, the p53 gene therapy in EOC with loss or mutation of p53 is thought to be the good strategy against intractable EOC with chemoresistance. In this regard, the therapeutic approach of p53 gene therapy should not be abandoned just because of the failure of the previous clinical trials. The failure could be the starting point from which to make improvements, through the careful analysis of the causes of failure. This review underscores the urgency of the development of effective strategies for control of epigenetic drivers of chemoresistance, among the plausible causes.

Epigenetic changes occurring during the acquisition of chemoresistance are substantial and complex. In addition, epigenetic changes might even outnumber genetic alterations.[34] It cannot be overemphasized that the identification of the epigenetic signatures of the platinum-resistant EOC is essential. The epigenetic signatures specific for the acquisition of chemoresistance can provide the information for the individualized epigenetic therapies. Technically, new validated tools to assess DNA methylation such as high-throughput quantitative analysis based on genome-wide array approaches will aid in the discovery of the critical methylation signatures, that can predict the outcomes of patients with EOC.[23] Regarding the treatment issues, many preclinical studies suggest that HDACIs exhibit enhanced anti-cancer activity in combination with demethylating agents, conventional chemotherapeutic agents, or other molecular targeted agents. Further studies are necessary for searching the combinations producing the best treatment outcomes in clinical settings.

CONCLUSIONS

Clear picture of epigenetic changes in EOC will provide a promising anticancer strategy. Tailored combinations of epigenetic modulators may reverse the chemoresistance and maximize the sensitization of tumor cells to conventional anti-cancer drugs. Elucidating the molecular epigenetic mechanisms of chemoresistance through further comprehensive researches is warranted.

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