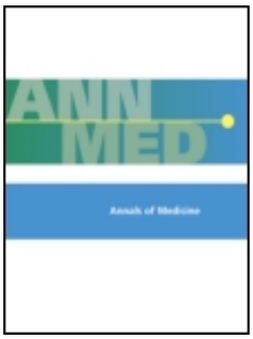

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: informahealthcare.com/journals/iann20

Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer

Jane Borley & Robert Brown

To cite this article: Jane Borley & Robert Brown (2015) Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer, Annals of Medicine, 47:5, 359-369, DOI: 10.3109/07853890.2015.1043140

To link to this article: https://doi.org/10.3109/07853890.2015.1043140

ANN MED

Taylor & Francis
Taylor & Francis Group

Annals of Medicine, 2015; 47: 359–369

ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.3109/07853890.2015.1043140

REVIEW ARTICLE

Epigenetic mechanisms and therapeutic targets of chemotherapy resistance in epithelial ovarian cancer

Jane Borley & Robert Brown

Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, London W12 0NN, UK

Epithelial ovarian cancer is the most lethal gynaecological cancer with the majority of patients succumbing to chemotherapy-resistant disease. Unravelling the mechanisms of drug resistance and how it can be prevented or reversed is a pivotal challenge in the treatment of cancer. Epigenetic mechanisms appear to play a crucial role in the development of inherent and acquired resistance in ovarian cancer. Aberrant epigenetic states can be reversed by drug therapy, and thus maintenance of epigenetic change is a potential target to halt or reverse chemotherapy resistance. This review explores the evidence that demonstrates that DNA methylation, histone modification, and microRNAs are associated with inherent and acquired chemotherapy resistance in ovarian cancer and the current challenges associated with this. We also explore current epigenetic therapies used in patients with drug-resistant ovarian cancer and future potential targets.

Key words: Chemotherapy resistance, DNA methylation, epigenetics, histone modification, microRNA, ovarian cancer

Key messages

- Epigenetic mechanisms are associated with chemotherapy resistance and have potential as clinical stratification biomarkers and therapeutic targets.
- Consistency in future studies is required to ensure a homogeneous sample and patient population with emphasis on relevant cell lines, samples, and clinical response.
- Epigenetic therapies have so far shown mixed benefit in patients with drug-resistant ovarian cancer mainly due to the side effect profile limiting drug delivery.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal of gynaecological malignancies, attributed to over 125,000 deaths per year worldwide (1) and over 4000 deaths in the UK alone in 2012 (2). Despite the improvement in both overall and progression-free survival since the advent of platinum-based agents, most women being treated for EOC eventually develop chemotherapy resistance. Understanding the mechanisms of this resistance, how to reverse resistant mechanisms, as well as the development of more targeted tumour-specific therapies that are active in chemotherapy-resistant disease is at the forefront of current EOC research strategy (3). There is growing evidence for a role for epigenetic mechanisms in acquired drug resistance (4–9), and this review will address the potential relevance of these mechanisms for EOC.

Initial chemotherapy response rates for standard regimes in EOC range from 60% to 75% (10,11), indicating that at least 20% of patients are resistant to first-line chemotherapy from the outset (platinum-refractory). In those that do respond, a proportion of patients will relapse within 6 months and are unlikely to respond again with the same treatment regime, indicating platinum-resistant disease. Second-line agents given to these patients have response rates of 30% at best (12). Those with a treatment-free interval of more than 12 months have a more favourable prognosis; however, the majority will eventually succumb to disease resistance to both platinum-based and other therapies within 5 years (2).

Chemotherapy resistance in ovarian cancer is probably due to a variety of mechanisms in a heterogeneous tumour cell population. This includes: 1) changes to pre-existing sensitive tumour cells which escape initial cytotoxic death and thereby become resistant, 2) survival of quiescent drug-tolerant cells (as cytotoxic agents are principally effective against proliferating cells), and 3) intrinsically resistant cells (for instance, tumour stem cells) which are present in relatively small numbers initially and then propagate as sensitive cells are destroyed (4,13). It has been proposed some time ago that ovarian cancer stem cells (OCSCs) can contribute to drug resistance and chemosensitive relapse of ovarian cancer (13), although experimental evidence to support this concept still is circumstantial (14). To date there are no definitive OCSC markers which identify ovarian OCSCs, and it remains unclear how OCSC markers relate to each other (14).

Correspondence: Professor Robert Brown, PhD, Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, London W12 0NN, UK. E-mail: b.brown@imperial.ac.uk

(Received 28 October 2014; accepted 15 April 2015)

Nevertheless epigenetic mechanisms such as elevated expression of histone methyltransferases occurring in putative OCSC have been suggested as leading to drug resistance, and poised epigenetic marks may have an important role in the evolution of drug resistance in ovarian cancer (15). Thus targeting epigenetic mechanisms associated with drug resistance may have merit in preventing the emergence of drug-resistant OCSC populations of cells. For example, resensitization to platinum-resistant cancer stem-like ALDH+ A2780 cells has recently been demonstrated using a DNA methyltransferase inhibitor (SGI-110) (16).

General mechanisms that contribute to primary or secondary chemotherapy resistance of EOC have been previously reviewed (13,17), and epigenetic regulation can occur at any number of these mechanistic pathways. Such mechanisms mainly are intratumoural; however, emerging evidence suggests that the host can also play a significant role in promoting therapy resistance (18). Recruitment of different host cell types to the treated tumour site occurs in response to a range of therapies. This host response may have a protective effect on the tumour cells, promoting a resistant tumour. A role for epigenetics in such mechanisms is still to be established, although DNA methylation variability in normal blood cells of patients has been suggested to be associated with response to chemotherapy in ovarian cancer (19). As epigenetic regulation can be potentially reversible through epigenetic therapies, there is potential in the delay of resistance or restoration of drug sensitivity in this approach. This review therefore summarizes the evidence to date on epigenetic mechanisms in EOC and makes recommendations for future studies.

Epigenetic mechanisms

The term 'epigenetics' has been defined as a heritable change in gene expression that is not due to an alteration in the DNA sequence (20); currently this includes various epigenetic processes such as histone modification, microRNA regulation, and DNA methylation. Epigenetic mechanisms are crucial for normal development and maintenance of cell type-specific responses. Histones are alkaline proteins which package DNA into structural units known as nucleosomes. Post-translational modifications such as acetylation, methylation, and phosphorylation occur on amino-terminal histone tails and are strongly associated with active gene transcription or transcriptional repression (21). Generally acetylation of histones by histone acetyltransferase (HAT) is associated with active genes, and hypoacetylation (by enzymes called histone deacetylases (HDACs)) with inactive regions (22). Histone methylation is associated with both active and silent genes, where, for instance, tri-methylation of lysine 27 of histone H3 (H3K27) is a silencing mark and methylation of lysine 4 (H3K4) is found at the promoters of active genes (23). DNA methylation is a process of addition of a methyl group to the position 5 carbon on the cytosine (C) nucleotide when followed by guanine (G) (CpG) in the presence of a family of enzymes known as DNA methyltransferase. DNA methyltransferases (DNMTs) perform the transfer of a methyl group from the endogenous co-factor S-adenosyl methionine (SAM or AdoMet) to the C5 position of the cytosine nucleotide. Traditionally, it was believed that this transfer is established via the *de novo* methyltransferases DNMT3A and DNMT3B and then maintained throughout cell division by DNMT1, due to its preference for hemi-methylated DNA (24). However, recently it has been suggested that DNMT3A and 3B may also have a role in maintenance (25). CpG islands (CGI) are defined as regions of the genome that contain a higher than expected frequency of CpG sites (normally 500 bp to 2 kb in length) (26). Approximately 70% of annotated gene promoters are associated with a CGI (27), and it has long been established that genes that are transcriptionally expressed are classically hypomethylated at CGIs while hypermethylation of CGIs is associated with transcriptional silencing (28,29). Epigenetic silencing of tumour suppressor genes (such as *BRCA1* and *APC*) is well recognized to be a contributing mechanism towards tumorigenesis in many cancer histotypes (22). Gene silencing may be caused by direct inhibition of transcription factor-binding or mediated by methyl-binding domain proteins that associate with the surrounding histone scaffolding (29–31). This subsequently recruits further complexes such as histone methyltransferases and HDACs which work together to compress chromatin into heterochromatin and thus 'close' transcription start sites by constricting the nucleosomes (32). The full cause or consequence of methylation which is not within the promoter region, such as intragenic methylation (IGM), is yet to be fully understood, although studies have demonstrated that genes with high IGM are expressed at higher levels (33,34). The hypotheses for this effect include: inhibition of the initiation of transcription from alternative transcription start sites (35), suppression of antisense strand mRNA or microRNA (36), and regulation of splicing (37).

MicroRNAs (miRNA) are a family of short (~22 nt) single-stranded ribonucleic acids which are also critically involved in gene expression. These molecules are non-protein coding and post-transcriptionally regulate gene expression through association with a multiprotein complex RNA-induced silencing complex (RISC). This complex then in turn typically binds at the 3' untranslated region of the target mRNA leading to translation inhibition, mRNA deadenylation, and decay (38). It has been predicted that over 30% of mRNA may be targeted by miRNAs, and therefore it is not surprising that a multitude of dysregulated miRNAs have been implicated in the development, behaviour, and progression of cancer (39–41).

Differential DNA methylation in association with chemotherapy resistance in ovarian cancer

Table I summarizes the studies to date that have investigated methylation of individual loci in direct association with either acquired or primary chemotherapy resistance in ovarian cancer cell lines or EOC tumour tissue. Hypermethylation at the promoter region of DNA-repair gene *hMLH1* (mutL homolog 1, colon cancer, non-polyposis type 2 (*E. coli*)) has been particularly widely studied in a variety of cancer subtypes including EOC. In platinum-resistant ovarian cancer cell lines (A2780 cisplatin-resistant clones) hypermethylation at the promoter region of *hMLH1* has been demonstrated when compared to sensitive cell lines (42). Importantly in this study, reversibility and resensitization to cisplatin is demonstrated with the addition of the demethylating agent decitabine. This differential methylation has also been observed in EOC patients with a significant increase in methylation at relapse and after four or more courses of platinum-based chemotherapy (43). Furthermore this differential methylation, which also predicts overall survival (OS), can be detected in cell-free circulating DNA from plasma of patients with EOC demonstrating its potential as a clinically relevant biomarker (44). Interestingly, in a separate study, there was no difference demonstrated in methylation of *hMLH1* in primary ovarian tumour samples comparing those sensitive to cisplatin to those intrinsically resistant, highlighting that that the biological mechanisms for intrinsic (primary) chemotherapy resistance can be separate from acquired (secondary) resistance (45).

Additional association studies have demonstrated hypermethylation of *BRCA1* to be associated with an increase in

Table I. Summary of studies investigating single gene methylation in relation to EOC chemotherapy resistance.

Author	Year	Gene name	Locus methylation in relation to drug resistance	Tissue source	Histological type	Chemotherapy response definition	Experimental model
Strathdee et al. (42)	1999	hMLH1	Hypermethylation	Cell lines (A2780 and resistant clones)	NA	NA	MSP
Gifford et al. (44)	2004	hMLH1	Hypermethylation	Plasma	Heterogeneous	South Western Oncology Group Solid Tumour Response Criteria or CA125	Fluorescent MSP
Strathdee et al. (5)	2005	MCJ	Hypermethylation	Tumour tissue	Heterogeneous	RECIST	Bisulphite sequencing
Teodoridis et al. (46)	2005	At least one of BRCA1, GSTp1, MGMT	Hypermethylation	Tumour tissue	Heterogeneous	South Western Oncology Group Solid Tumour Response Criteria or CA125	MSP
Watanabe et al. (43)	2007	hMLH1	Hypermethylation	Tumour tissue	Heterogeneous, paired samples	WHO criteria	MSP
Staub et al. (86)	2007	Hsulf-1	Hypermethylation	Cell line (OV207, SKOV3)	NA	NA	Bisulphite sequencing
Nicholson et al. (87)	2009	ASS1	Hypermethylation	Cell line (A2780, A2780 CisR), tumour tissue	Heterogeneous (80% serous)	RECIST	MSP
Su et al. (88)	2009	SFRP5	Hypermethylation	Tumour tissue	Heterogeneous	CT and CA125	MSP
Bram et al. (89)	2009	ABCG2	Hypomethylation	Cell line (IGROV1, IGROV1/T8)	NA	NA	COBRA
Chaudhry et al. (47)	2009	BRCA1	Hypomethylation	Tumour tissue	Heterogeneous (86% serous)	CT and CA125	MSP
Dai et al. (56)	2011	DVL1, NFATC3	Hypermethylation	Tumour tissue	Heterogeneous	RECIST	DMH array
Iramaneerat et al. (90)	2011	HERV-K	Hypomethylation	Tumour tissue	Clear cell	Not specified	COBRA
Syed et al. (91)	2011	Plk2	Hypermethylation	Cell line (SKOV3, A2780, and resistant clones)	NA	NA	MSP and bisulphite sequencing
Wang et al. (48)	2012	TGFBI	Hypermethylation	Cell line (SKOV3, A2780, and resistant clones)	NA	NA	MSP and bisulphite sequencing
Coley et al. (49)	2012	p57kip2	Hypermethylation	Cell line (PEO1 and resistant clones)	NA	NA	MSP and pyrosequencing
Ali et al. (92)	2013	RGS10-1	Hypermethylation	Cell line (A2780 and resistant clone)	NA	NA	Bisulphite sequencing

clinical response to chemotherapy in ovarian tumours (46,47). Differential methylation of transforming growth-factor-beta inducible gene-h3 (*TGFBI*) (48) and $p57^{Kip2}$ (49) has also been associated with platinum-resistant cell lines, and hypermethylation of methylation-controlled *DNA J* (*MCJ*) gene was associated with poor chemotherapy response and decreased OS in EOC tumours (5). There are several limitations to these studies as those involving patient material often use heterogeneous histology, now well recognized to be molecularly different and as such should be regarded as different disease entities (3). In addition, these studies often use variable definitions of chemotherapy response, making the summation of data challenging. The majority of the early studies use methylation-specific PCR (MSP) to determine DNA methylation. This process allows for specific investigation of customized loci at small quantities of DNA, which is qualitative and semi-quantitative (50) but is prone to false positives and not suitable for investigation of large numbers of methylation loci. Pyrosequencing technology is now widely used for single locus analysis, is a robust assay that allows methylation to be quantitated, and has the added benefit that it is suitable for detecting differential DNA methylation in minute amounts of DNA within body fluids (51).

More recently the advancement in DNA methylation technology has allowed a genome-wide analysis of differential methylation in association with chemotherapy resistance. The earliest study (52) used a custom differential methylation hybridization (DMH) array and Affymetrix U133 gene expression array to compare A2780 sensitive clones to isogenic resistant clones, developed over a variety of cisplatin exposures, to identify chemoresistance-associated loci. There was a demonstrated increase in hypermethylated genes dependent on number of treatment exposures and a significant correlation between the total number of methylated genes and the IC50 of the resistant sub-lines. In keeping with this there was a significant increase in expression of DNA methyltransferases DNMT1 and DNMT3B in resistant sublines. Furthermore, treating the resistant clone with DNA methyltransferase inhibitors, decitabine, and zebularine demonstrated a dose-dependent decrease in IC50 and increase in cisplatin sensitivity. In a similar study using the Infinium HumanMethylation27 Beadchip and Affymetrix U133 gene expression array differential methylation and gene expression were determined between A2780 and A2780 cisplatin-resistant clones (6). A total of 4092 genes were hypermethylated at more than one CpG site in the resistant clones, whereas only 1289 genes were hypomethylated. From the 4092 hypermethylated genes, 245 genes were found to be down-regulated on the gene expression array. Treatment of the resistant clone A2780/cp70 with decitabine induced re-expression of 41 of the 245 down-regulated genes. These findings were also validated by pyrosequencing in cell line models of *in vivo* cisplatin resistance and relapsed tumour samples with three genes, *ARMCX2*, *MEST*, and *MLH1*, consistently having higher methylation in resistant samples. One further study investigated A2780 versus *in vitro*-derived cisplatin-resistant clones using Methyl-Capture sequencing (MethylCap-seq) which identified 1224 hypermethylated and 1216 hypomethylated differentially methylated regions (53). In contrast to the previous studies the authors found a lower global methylation in resistant lines compared to sensitive lines; however, the differences were mostly found at intragenic regions which are not well represented on DMH and Infinium HumanMethylation27 arrays. It should be noted that in both of these studies the A2780 cell line and its parenteral resistant clones were used as a model for ovarian cancer, in keeping with many other published studies. However, there is now good evidence that this cell-line is not an appropriate model for high-grade serous cancer and may be more similar to endometrial ovarian cancer and even closely related to lung, liver, and gastric cancer tumours (54).

Studies directly investigating DNA methylation in EOC tumour samples (as opposed to cell lines) are sparse. One such study uses a DMH array to determine differential DNA methylation on 36 advanced-stage serous ovarian cancer samples. This demonstrated 749 loci whose methylation was significantly different between patients with refractory or resistant disease (defined as disease progression through or less than 6 months from platinum treatment) versus those termed late sensitive (relapse after 12 months of platinum treatment) (55). Of the differentially methylated loci, approximately 60% of samples were methylated in resistant tumours compared to 40% in sensitive tumours. The candidate methylation loci were then matched to a gene expression array, and 296 genes were identified which demonstrated a difference in gene expression in association with methylation status. These 296 target genes were then further selected by using a shRNA screen in a carboplatin resistance assay on resistant ovarian cancer cell lines. From this, 19 genes were identified that when supressed altered the platinum resistance of the cell lines, including *FZD1* (an important Wnt-signalling receptor). A separate study, principally performed to investigate the association of DNA methylation in Wnt pathway genes (determined by DMH array) to progression-free survival in 120 primary EOC tumours, also demonstrated that increased methylation of *DVL1* and *NFATC3* correlated to poor primary platinum-based chemotherapy response (56). It was observed that for every unit increase of methylation *Z* score the odds ratio (OR) of the patients with progressive or stable disease to the patients with partial or complete response was 1.7 (95% CI 1.1–2.8, $P = 0.026$, false discovery rate (FDR) $< 10\%$) for *DVL1* and 1.6 (95% CI 1.0–2.6, $P = 0.032$, FDR $< 10\%$) for *NFATC3*. These findings were replicated in The Cancer Genome Atlas (TCGA) data set and, in keeping with the association of promoter hypermethylation and gene inactivation, the investigators found a significant inverse correlation with gene expression data. Furthermore a decrease in expression of *DVL1* was found to be significantly associated with poor chemotherapy response in the TCGA cohort (OR 0.5, 95% CI 0.3–0.9, $P = 0.035$) (56).

Overall these studies demonstrate association with differential DNA methylation and chemotherapy resistance or response. However, it is often unclear as to whether these loci are a driver of resistance (whereby the differential methylation causes transcriptional changes which contribute to resistance) or simply a consequence of separate unknown mechanisms which coincidentally cause an alteration in methylation. For example, methylation on Lys27 of histone H3 appears to pre-mark genes for *de novo* DNA methylation in cancer (57). In addition difficulties in separating the cellular components within tumour tissue leads to a global estimation of methylation rather than a cell-specific quantity. Further validation of target loci is required for any loci to be clinically meaningful, with consensus agreements needed on the number of CpG sites to be sampled and the ideal experimental platform.

Histone modifications in association with chemotherapy resistance in ovarian cancer

There is less available evidence of the role of histone modifications at specific loci associated with EOC resistance, but this is perhaps due to the technical difficulties of determining histone modification (in comparison to DNA methylation, for example). Nevertheless, as recently reviewed, this is an important future area for further investigation in drug resistance using emerging next-generation sequencing technology and epigenetic editing (4). One of the earliest reports demonstrated that over-expressing

a dominant negative histone transgene was able to reduce global levels of H3K27me in cisplatin-resistant A2780/cp70 cells and led to resensitization and a 4-fold reduction in the cisplatin IC50 (58). This change was thought to be in part due to altered gene expression, with an up-regulation of *MLH1* and *RASSF1A* amongst others. There is growing evidence that both repressive and permissive histone modifications can occur at the same time on the same gene promoter, which can then be considered to be in a bivalent state, poised for activation or repression of cancer cells. This then in turn leads to stable epigenetic changes which depend on activation, for example through exposure to chemotherapy leading to stable acquired resistant cells (59). Our group has demonstrated gene sets associated with bivalent H3K27me3 and H3K4me3 in high-grade serous ovarian cancer (HGSOC) patient tumours which are significantly differentially expressed in the cisplatin-acquired resistant HGSOC ovarian cell line PEO4 versus the chemotherapy-sensitive line PEO1 (60).

Further evidence for the role of histone modification in ovarian cancer includes EZH2, a specific H3K27 methyltransferase found to be over-expressed in cisplatin-resistant A2780/DDP cells compared to sensitive A2780 (61). Furthermore a loss of EZH2 through shRNA transfection was shown to resensitize resistant cells to cisplatin in *in vitro* and *in vivo* models.

It is likely that a combination of different histone modifications and DNA methylation interplay in the development of chemotherapy resistance, and future studies should explore this further.

MicroRNA in association with chemotherapy resistance in ovarian cancer

A plethora of studies have found associations with miRNAs and resistant ovarian cell lines. Those which have been validated by either *in vitro* or *in vivo* knockdown/re-expression sensitivity assays or in patient tumour tissue are summarized in Table II. Most studies use ovarian cancer cells lines; very few validate findings in clinical material. Four studies use an exploratory approach in a heterogeneous mix of EOC tumour tissue (62–65). They utilize miRNA array platforms to generate miRNA signatures dependent on chemotherapy response. Table III summarizes the findings from these four studies. Adequate FDR correction analysis which is crucial in analysis of large data sets (66) is lacking in three of four studies, with only one study using a FDR cut-off of $< 10\%$ (65). There is poor reproducibility between studies, although this may be partly explained by the different microarray platforms used and different categorization of chemotherapy response. The two most recent studies make efforts to perform test validation in analysis. Bagnoli et al. (65) used the Illumina miRNA BeadChips Array to determine differential expression of miRNAs in 55 advanced-stage EOC tumours. With a FDR of $< 10\%$, an expression signature consisting of 18 down-regulated and 14 up-regulated miRNAs in patients with early relapse (time to progression less than 12 months) was generated. A total of 10 miRNAs remained significant in a validation set ($n = 30$), 9 of which were located at chrXq27.3; the authors summarized that these findings represented a 'highly correlated and co-expressed miRNA cluster'. Furthermore, unsupervised hierarchical clustering based on the miRNA signature correctly classified the validation set according to relapse in 90% of cases. Vecchione et al. (63) determined a separate miRNA signature with 23 differentially expressed miRNAs, from 86 EOC tumour samples, using the TaqMan Array Human MicroRNA Set. Cluster analysis determined samples grouped into those that responded to chemotherapy (RECIST complete and partial response) and those that did not (RECIST stable disease and progressive disease). Validation of this signature was attempted in an independent set of 112 samples using the TaqMan MicroRNA assay with three miRNAs, mir-484, mir-642, and mir-217, remaining significant in ANOVA analysis.

The lack of reproducibility between these studies suggests possible deficiencies in study design especially in regard to histotype of EOC and lack of statistically significant validation and FDR correction. Future biomarker studies should focus on ensuring adequate power within the experimental design, remaining within REMARK criteria (67) and ensuring that the histotype of tissue is accurately represented.

Targeting chemotherapy resistance through epigenetic therapies

Unlike genetic mutations, DNA methylation and histone modifications are reversible and are thus important targets for effective cancer treatment. Indeed both 5-azacytidine and decitabine (5-aza-2'-deoxycytidine), both demethylating agents, are currently used in clinical practice for myelodysplastic syndrome (MDS) and cutaneous T cell lymphoma. These drugs, classified as DNMT inhibitors, exert their demethylating activity by being incorporated into the DNA of S-phase cells in the place of cytosine. Covalent bonds are subsequently formed with DNMT, resulting in a reduction of the active enzyme and a subsequent loss of methylation (68). In MDS patients, the use of DNMT inhibitors has been shown to improve quality of life, significantly improve OS (69), and have led to complete remission rates of up to 39% (70). Numerous *in vitro* and *in vivo* studies have demonstrated that the addition of DNMT inhibitors and HDAC inhibitors can reverse acquired drug resistance (42,48,49,52,71,72). These drugs have since translated to the clinical setting in phase 1 and 2 trials for patients with resistant disease in solid malignancies.

Demethylating agent clinical studies

Specifically in relation to ovarian cancer, phase 1 studies have proven the safety of DNMT inhibitors, albeit with common toxicities of allergy, rash, and gastrointestinal disturbances (73,74). Myelosuppression toxicities are also closely correlated to dose escalation (75). Importantly for these phase 1 studies, demethylation has been demonstrated at clinically acceptable doses in peripheral blood mononuclear cells (PBMCs), cell-free circulating DNA in plasma, and tumour biopsies, proving the mechanistic action of the drugs (73,75). Three phase 2 clinical trials have so far been published. The earliest published study (76,77) randomized patients with relapse of EOC within 6–12 months of platinum treatment to either six cycles of carboplatin (AUC 6) or 90 mg/m^2 decitabine on day 1 and carboplatin on day 8. The dose of decitabine had to be reduced to 45 mg/m^2 after the first four enrolled patients had frequent dose delays due to neutropenia. Despite this dose reduction none of the patients in the decitabine arm were able to complete six cycles of treatment due to hypersensitivity reactions and neutropenia. Additionally there was no RECIST response in this group compared to 6/14 responses in the carboplatin-only group. The trial therefore closed early. A separate study (phase 1b–2a) (78) selected high-grade EOC patients with platinum-refractory or resistant disease (relapse within 6 months) to receive subcutaneous azacitidine 75 mg/m^2 daily for 5 days and carboplatin (AUC 4 or 5) on day 2. From 29 evaluable patients, 17 received six or more cycles, and no dose-limiting toxicities or treatment-related deaths were observed. Clinical chemotherapy response was defined by WHO criteria, 1 patient had complete response, 3 patients had partial response, and 10 patients had stable disease, which is particularly encouraging for patients with refractory disease. One further study (79) recruited patients with

Table II. Summary of validated miRNA associated with EOC chemotherapy resistance.

Author	Year	MicroRNA ID	Regulation in resistant models	Gene target	Platform	Initial identifying tissue	Validation in tumour	Functional *in vitro* / *in vivo* validation
Yang et al. (93)	2008	mir-214	up	PTEN	RT-PCR	cell lines	✓	✓
Yang et al. (64)	2008	let-7i	down		Customized array	tumour tissue	✓	✓
		34 miRNA differentially expressed						
Li et al. (94)	2010	miR-27a	up	HIPK2	RT-PCR	cell lines	×	✓
Ye et al. (95)	2010	miR-376c	up	ALK7	RT-PCR	cell lines	✓	✓
Bagnoli et al. (65)	2011	chrXq27.3 cluster	up		miRNA BeadChip Array (Illumina)	tumour tissue	✓	✓
Kong et al. (96)	2011	miR-125b	up	Bak1	RT-PCR	cell lines	×	✓
Mateescu et al. (97)	2011	MiR-141	down	p38alpha	RTPCR	cell lines	×	validation study
		miR-200a	down	p38alpha				
Boyerinas et al. (98)	2012	let-7	down		RTPCR	cell lines	×	validation study
Cheng et al. (99)	2012	miR-199a	up		RT-PCR	cell line	×	validation study
Cittelly et al. (100)	2012	miR-200c	down		RT-PCR	cell line	×	validation study
Fu et al. (101)	2012	miR-93	up	PTEN	Customized microarray	cell line	✓	✓
Yang et al. (102)	2012	miR-130b	down	CSF-1	RT-PCR	cell lines	×	✓
Yang et al. (103)	2012	miR-130a	up	MDR1	miRCURY LNA Array (Exiqon)	cell line		✓
		89 miRNA differentially expressed					×	
Ziliak et al. (104)	2012	miR-193b	up	CRIM1	miRURY LNA Array (Exiqon)	cell lines	×	✓
		miR-320a						
Cai et al. (105)	2013	Let-7e	down	EZH2, CCND1	RT-PCR	cell lines	×	✓
Huh et al. (106)	2013	mir-106a	up	caspase-7, BCL10	miRNA-V1 BeadChips (Illumina)	cell line	✓	✓
		mir-591	down	ZEB1			×	✓
		10 miRNA differentially expressed						
Li et al. (107)	2013	miR-320a	up	RAB34	Exiqon microarray, RT-PCR	cell line	✓	×
		miR-22	up					
		mir-129-5p	up					
		miR-9	down					
		miR-155	down					
		miR-640	down					
van Jaarsveld et al. (108)	2013	miR-141	up	KEAP1	LNA-based capture probe set (Exiqon)	cell line	×	✓
		miR-200c	up					
		27 miRNA differentially expressed						
Prislei et al. (109)	2013	mir-200c	down	TUBB3	RT-PCR	cell line	×	✓
Rao et al. (110)	2013	mir-106a	down	Mcl-1	RT-PCR	cell lines	×	✓
Vecchione et al. (63)	2013	mir-484	down	VEGFB and VEGFR2	TaqMan microRNA assay	tumour tissue	✓	✓
		mir-642	down					
		mir-217	down					
Zhang et al. (111)	2013	miR-130a	down	XIAP	RT-PCR	cell line	×	✓
Xiang et al. (112)	2013	miR-152	down	DNMT1	RT-PCR	cell line	×	✓
		miR-185	down	DNMT1				
Yu et al. (113)	2014	mir-29a/b/c	down	COL1A1	RT-PCR	cell line	×	✓
Chan et al. (114)	2014	miR-21	up	PDCD4, c-IAP2	Exiqon microarray, RT-PCR	cell line	✓ (TCGA)	✓
Chen et al. (115)	2014	miR- 367	down	Multiple	Customized array, RT-PCR	cell line	×	✓
		miR- 30a-5p	up					
Li et al. (116)	2014	miR-128	down	ABCC5, Bmi-1	RT-PCR	cell line	×	✓
Li et al. (117)	2014	miR-106a	up	PDCD4	RT-PCR	cell line	×	✓
Zhu et al. (118)	2014	miR-145	down	Sp1, Cdk6	RT-PCR	tumour tissue	✓	✓
Zong et al. (119)	2014	miR-130b	up	MDR1	RT-PCR	cell line	×	✓

Table III. Summary of four independent studies associating miRNA expression and chemotherapy response in clinical material.

Study	miRNA ID	Expression in relation to resistance	FDR correction
Yang et al. 2008	let-7i	up	0.67
	mif-216	down	0.67
	mir-106b	up	0.69
	mir-123	down	0.67
	mir-126	down	0.67
	mir-129	down	0.67
	mir-140	down	0.67
	mir-146	down	0.67
	mir-148	down	0.67
	mir-152	down	0.67
	mir-181c	down	0.67
	mir-196a	down	0.67
	mir-198	down	0.67
	mir-1b-1	down	0.67
	mir-203	up	0.67
	mir-214	down	0.67
	mir-22	down	0.67
	mir-223	down	0.67
	mir-29a	down	0.67
	mir-320	down	0.67
	mir-320	down	0.67
	mir-321	up	0.67
	mir-365	down	0.67
	mir-370	down	0.67
	mir-452	down	0.67
	mir-453	up	0.67
	mir-491	up	0.67
	mir-507	up	0.67
	mir-509	up	0.67
	mir-513	up	0.67
	mir-514	up	0.67
	mir-519e	down	0.67
	mir-520e	down	0.67
	mir-521	down	0.67
Eitan et al. 2009	let-7g	up	>0.3
	mir-23a	up	0.3
	mir-27a	up	0.3
	mir-30c	up	>0.3
	mir-378	down	0.3
	mir-625	down	>0.3
	mirr-199a-3p	up	>0.3
Vecchione at al. 2013	mir-181a	down	Not corrected
	mir-19a	up	Not corrected
	mir-217	down	Not corrected
	mir-302d	down	Not corrected
	mir-483-5q	down	Not corrected
	mir-484	down	Not corrected
	mir-491	down	Not corrected
	mir-592	up	Not corrected
	mir-642	down	Not corrected
	mir-653	down	Not corrected
	mir-671-3p	down	Not corrected
	mir-744	down	Not corrected
Bagnoli et al. 2011	HS-138	down	<0.1
	HS-141	down	<0.1
	let-7f-1	up	<0.1
	mir-139-5p	up	<0.1
	mir-15b	up	<0.1
	mir-188-5p	down	<0.1
	mir-191	down	<0.1
	mir-202	down	<0.1
	mir-202	down	<0.1
	mir-22	up	<0.1
	mir-27b	up	<0.1
	mir-299-5p	up	<0.1
	mir-30c-2	up	<0.1
	mir-32	down	<0.1
	mir-339-3p	down	<0.1
	mir-411	up	<0.1
	mir-454	up	<0.1

(*Continued*)

Table III. (Continued)

Study	miRNA ID	Expression in relation to resistance	FDR correction
	mir-485-3p	up	<0.1
	mir-493	up	<0.1
	mir-494	up	<0.1
	mir-506	down	<0.1
	mir-507	down	<0.1
	mir-508-3p	down	<0.1
	mir-509-3-5p	down	<0.1
	mir-509-3p	down	<0.1
	mir-509-5p	down	<0.1
	mir-513a-3p	down	<0.1
	mir-513a-5p	down	<0.1
	mir-513b	down	<0.1
	mir-514	down	<0.1
	mir-656	up	<0.1
	solexa-499–2217	up	<0.1

Bold text = significant in training and test set.

platinum-refractory EOC disease. Treatment consisted of decitabine 10 mg/m^2 intravenously for 5 days and carboplatin on day 8 (AUC 5). Altogether 17 patients enrolled into the study, with most patients receiving six cycles of treatment. Grade 3–4 toxicities included neutropenia ($n = 4$) and thrombocytopenia ($n = 2$). From 17 patients, 1 had RECIST defined complete response, 5 had partial response, and 6 had stable disease which lasted for more than 3 months. The authors concluded that the improved side effect profile in this study compared to the previously similar study (80) was due to the lower dose of decitabine administered and the use of routine growth factor support (peg-filgastrim) to prevent prolonged myelosuppression. Furthermore global and gene specific methylation was proven in PBMC, ascites, and tumour DNA at this lower dose.

Histone modification clinical studies

Three studies have investigated the efficacy of HDAC inhibitors in EOC patients with resistant or refractory disease in phase 2 clinical trials (81–83). The first of these used single-agent belinostat on day 1 to 5 of a 21-day cycle in patients with platinum-resistant disease (PFS within 6 months of platinum therapy) (83). Of 18 patients, 15 showed response, with 9 (60%) demonstrating stable disease, and 6 (40%) with progressive disease, although all patients were off-study by the end of analysis. In addition, as a platinum agent was not given in combination with belinostat, it is unclear whether belinostat causes reversal of platinum resistance. Two more recent studies have more specifically investigated the use of belinostat in patients with resistant disease in combination with platinum-based agents. Dizon et al. (81) combined belinostat day 1 to 5 with carboplatin (administered 2–3 hours after day 3 belinostat) in patients with platinum-resistant disease (Progression Free Survival, PFS, <6 months from treatment). A total of 27 eligible patients were recruited and received a median number of two treatment cycles. One patient demonstrated complete response, 1 partial response, and 12 patients (44.4%) had stable disease. As the overall response rate was 7.4%, the authors concluded that belinostat did not improve the activity of carboplatin in this resistant population. Following this, a similar trial added paclitaxel to the belinostat/carboplatin combination and recruited 35 patients with EOC (82), 16 of whom had progressed within 6 months of cisplatin/taxane treatment, and 19 of whom progressed after 6 months. With this combination the overall response rate appeared much improved at 44% in those with platinum-resistant disease.

Other potential therapeutic targets which are currently less well developed include histone methyltransferase inhibitors that prevent gene silencing through inhibition of histone trimethylation (84). Additionally the benefits of combining DNA demethylating agents and HDAC inhibitors have been proposed to be acting synergistically to 'unlock and open' the gene which has become epigenetically silenced (68,71,85). Further clinical approaches to epigenetic drug development include using single agents to switch on tumour suppressor genes fundamental to particular cancer development, maintenance therapy to prevent relapse or resistance following a course of conventional treatment, and prophylaxis to patients at high risk of developing disease such as those found through epigenetic risk biomarkers (85). Several concerns about the safety of epigenetic therapies include the unknown and non-specific effects on normal tissue, the high side effect profile, and the potential carcinogenic effect. However, it is important to recognize that the majority of these treatments are being trialled as second- or third-line drugs, when conventional chemotherapy has already failed and treatment options for the patient and the medical team are extremely limited. In addition there may be several mechanisms for why, at present, epigenetic therapies are less effective in solid tumours in comparison to haematological malignancies. This includes drug delivery and targeting (achieving the optimal dose at the specific sites whilst minimizing side effects peripherally), the relative lower number of proliferating cells in solid tumours, and the need to eradicate tumour stem cell populations (85).

Summary

Strong associations clearly exist between epigenetic marks and chemotherapy resistance mechanisms in both cell lines and EOC tumour samples, and it is evident that epigenetics has a part to play in cancer resistance. However, the complexity and heterogeneity of these mechanisms and their interaction make interpretation difficult. Previous studies have suffered in quality due to the use of the heterogeneous tumour samples or lack of consistency in the definitions of chemotherapy response. It is also now well recognized that EOC is not one disease entity and that these tumours are particularly heterogeneous with distinct molecular, biological, aetiological, and clinical profiles (3). Therefore future studies should not group heterogeneous tissue types together, and most will focus on the commonest form of EOC, high-grade serous ovarian cancer. Many studies use cell lines which now appear to be a poor model of ovarian cancer or do not validate significant findings in independent data sets. Stringent criteria as demonstrated in prognostic biomarker research through REMARK criteria

(67) should also be applied to future work to ensure high-quality association studies. There is also an additional complexity of determining differences between primary intrinsic drug resistance and secondary acquired drug resistance, and there is a need for high-quality longitudinal studies with paired sequential samples to determine these changes. In the future, data generated from patient samples obtained from the British Translational Research Ovarian Cancer Collaborative (BriTROC) will hopefully address some of these issues.

It is also vital to determine whether associations of individual targets and loci are drivers of resistance and thus potential targets or purely passengers of resistance with no direct biological action or a consequent contribution to the resistance mechanism itself. In addition, whether these associations in a heterogeneous cell population within the tumour mass can be clinically validated still requires addressing. Future work in the field of epigenetics aims to answer these questions, with the intention to use these tools to discover reproducible biomarkers to identify accurately those with resistant tumours. This will ultimately aid clinical management decisions as well as advancing epigenetic drug development to prevent or reverse these resistant mechanisms.

Declaration of interest: The authors have no disclosures of interest.

References

1. Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. Best Pract Res Clin Obstet Gynaecol. 2006;20:207–25.
2. Cancer Research UK. CancerStats: Ovarian cancer - UK. 2015. Available at: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/ovary/.
3. Vaughan S, Coward JI, Bast RC, Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer. 2011;11:719–25.
4. Brown R, Curry E, Magnani L, Wilhelm-Benartzi CS, Borley J. Poised epigenetic states and acquired drug resistance in cancer. Nat Rev Cancer. 2014;14:747–53.
5. Strathdee G, Vass JK, Oien KA, Siddiqui N, Curto-Garcia J, Brown R. Demethylation of the MCJ gene in stage III/IV epithelial ovarian cancer and response to chemotherapy. Gynecol Oncol. 2005;97:898–903.
6. Zeller C, Dai W, Steele NL, Siddiq A, Walley AJ, Wilhelm-Benartzi CS, et al. Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. Oncogene. 2012;31:4567–76.
7. Marsh DJ, Shah JS, Cole AJ. Histones and their modifications in ovarian cancer - drivers of disease and therapeutic targets. Front Oncol. 2014;4:144.
8. Shukla S, Meeran SM. Epigenetics of cancer stem cells: pathways and therapeutics. Biochim Biophys Acta. 2014;1840:3494–502.
9. Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell. 2014;54:716–27.
10. Sandercock J, Parmar MK, Torri V, Qian W. First-line treatment for advanced ovarian cancer: paclitaxel, platinum and the evidence. Br J Cancer. 2002;87:815–24.
11. Neijt JP, Engelholm SA, Tuxen MK, Sorensen PG, Hansen M, Sessa C, et al. Exploratory phase III study of paclitaxel and cisplatin versus paclitaxel and carboplatin in advanced ovarian cancer. J Clin Oncol. 2000;18:3084–92.
12. Edwards SJ, Barton S, Thurgar E, Trevor N. Topotecan, pegylated liposomal doxorubicin hydrochloride, paclitaxel, trabectedin and gemcitabine for advanced recurrent or refractory ovarian cancer: a systematic review and economic evaluation. Health Technol Assess. 2015;19:1–480.
13. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer. 2003;3:502–16.
14. Ffrench B, Gasch C, O'Leary JJ, Gallagher MF. Developing ovarian cancer stem cell models: laying the pipeline from discovery to clinical intervention. Mol Cancer. 2014;13:262.
15. Rizzo S, Hersey JM, Mellor P, Dai W, Santos-Silva A, Liber D, et al. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. Mol Cancer Ther. 2011;10:325–35.
16. Wang Y, Cardenas H, Fang F, Condello S, Taverna P, Segar M, et al. Epigenetic targeting of ovarian cancer stem cells. Cancer Res. 2014;74:4922–36.
17. Davis A, Tinker AV, Friedlander M. "Platinum resistant" ovarian cancer: what is it, who to treat and how to measure benefit? Gynecol Oncol. 2014;133:624–31.
18. Katz OB, Shaked Y. Host effects contributing to cancer therapy resistance. Drug Resist Updat. 2015;19:33–42.
19. Flanagan JM, Wilhelm-Benartzi CS, Metcalf M, Kaye SB, Brown R. Association of somatic DNA methylation variability with progression-free survival and toxicity in ovarian cancer patients. Ann Oncol. 2013;24:2813–18.
20. Holliday R. The inheritance of epigenetic defects. Science. 1987;238:163–70.
21. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell. 2012;150:12–27.
22. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer. 2011;11:726–34.
23. Sawan C, Herceg Z. Histone modifications and cancer. Adv Genet. 2010;70:57–85.
24. Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem. 2011;12:206–22.
25. Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nat Rev Genet. 2009;10:805–11.
26. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nat Rev Genet. 2011;12:529–41.
27. Saxonov S, Berg P, Brutlag DL. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. Proc Natl Acad Sci U S A. 2006;103:1412–17.
28. Bird AP. CpG-rich islands and the function of DNA methylation. Nature. 1986;321:209–13.
29. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13:484–92.
30. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011;25:1010–22.
31. Bogdanović O, Veenstra GJ. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. Chromosoma. 2009;118:549–65.
32. Mazzio EA, Soliman KF. Basic concepts of epigenetics: impact of environmental signals on gene expression. Epigenetics. 2012;7:119–30.
33. Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. Nat Genet. 2007;39:61–9.
34. Aran D, Toperoff G, Rosenberg M, Hellman A. Replication timing-related and gene body-specific methylation of active human genes. Hum Mol Genet. 2011;20:670–80.
35. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010;466:253–7.
36. Tufarelli C, Stanley JA, Garrick D, Sharpe JA, Ayyub H, Wood WG, et al. Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. Nat Genet. 2003;34:157–65.
37. Laurent L, Wong E, Li G, Huynh T, Tsirigos A, Ong CT, et al. Dynamic changes in the human methylome during differentiation. Genome Res. 2010;20:320–31.
38. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15:509–24.
39. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet. 2007;39:673–7.
40. Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer. 2007;43:1529–44.
41. Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer - a brief overview. Adv Biol Regul. 2015;57:1–9.
42. Strathdee G, MacKean MJ, Illand M, Brown R. A role for methylation of the hMLH1 promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. Oncogene. 1999;18:2335–41.
43. Watanabe Y, Ueda H, Etoh T, Koike E, Fujinami N, Mitsuhashi A, et al. A change in promoter methylation of hMLH1 is a cause of acquired resistance to platinum-based chemotherapy in epithelial ovarian cancer. Anticancer Res. 2007;27(3b):1449–52.

44. Gifford G, Paul J, Vasey PA, Kaye SB, Brown R. The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients. Clin Cancer Res. 2004;10:4420–6.
45. Helleman J, van Staveren IL, Dinjens WN, van Kuijk PF, Ritstier K, Ewing PC, et al. Mismatch repair and treatment resistance in ovarian cancer. BMC Cancer. 2006;6:201.
46. Teodoridis JM, Hall J, Marsh S, Kannall HD, Smyth C, Curto J, et al. CpG island methylation of DNA damage response genes in advanced ovarian cancer. Cancer Res. 2005;65:8961–7.
47. Chaudhry P, Srinivasan R, Patel FD. Utility of gene promoter methylation in prediction of response to platinum-based chemotherapy in epithelial ovarian cancer (EOC). Cancer Invest. 2009;27:877–84.
48. Wang N, Zhang H, Yao Q, Wang Y, Dai S, Yang X. TGFBI promoter hypermethylation correlating with paclitaxel chemoresistance in ovarian cancer. J Exp Clin Cancer Res. 2012;31:6.
49. Coley HM, Safuwan NA, Chivers P, Papacharalbous E, Giannopoulos T, Butler-Manuel S, et al. The cyclin-dependent kinase inhibitor p57(Kip2) is epigenetically regulated in carboplatin resistance and results in collateral sensitivity to the CDK inhibitor seliciclib in ovarian cancer. Br J Cancer. 2012;106:482–9.
50. Herman JG, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A. 1996;93:9821–6.
51. Paliwal A, Vaissiere T, Herceg Z. Quantitative detection of DNA methylation states in minute amounts of DNA from body fluids. Methods. 2010;52:242–7.
52. Li M, Balch C, Montgomery JS, Jeong M, Chung JH, Yan P, et al. Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. BMC Med Genomics. 2009;2:34.
53. Yu W, Jin C, Lou X, Han X, Li L, He Y, et al. Global analysis of DNA methylation by Methyl-Capture sequencing reveals epigenetic control of cisplatin resistance in ovarian cancer cell. PLoS One. 2011;6:e29450.
54. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun. 2013;4:2126.
55. Lum E, Vigliotti M, Banerjee N, Cutter N, Wrzeszczynski KO, Khan S, et al. Loss of DOK2 induces carboplatin resistance in ovarian cancer via suppression of apoptosis. Gynecol Oncol. 2013;130:369–76.
56. Dai W, Teodoridis J, Zeller C, Graham JS, Hersey JM, Flanagan JM, et al. Systematic CpG islands methylation profiling of genes in the Wnt pathway in epithelial ovarian cancer identifies biomarkers of progression-free survival. Clin Cancer Res. 2011;17:4052–62.
57. Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet. 2007;39:232–6.
58. Abbosh PH, Montgomery JS, Starkey JA, Novotny M, Zuhowski EG, Egorin MJ, et al. Dominant-negative histone H3 lysine 27 mutant derepresses silenced tumor suppressor genes and reverses the drug-resistant phenotype in cancer cells. Cancer Res. 2006;66:5582–91.
59. Brown R, Curry E, Magnani L, Wilhelm-Benartzi CS, Borley J. Poised epigenetic states and acquired drug resistance in cancer. Nat Rev Cancer. 2014;14:747–53.
60. Chapman-Rothe N, Curry E, Zeller C, Liber D, Stronach E, Gabra H, et al. Chromatin H3K27me3/H3K4me3 histone marks define gene sets in high-grade serous ovarian cancer that distinguish malignant, tumour-sustaining and chemo-resistant ovarian tumour cells. Oncogene. 2013;32:4586–92.
61. Hu S, Yu L, Li Z, Shen Y, Wang J, Cai J, et al. Overexpression of EZH2 contributes to acquired cisplatin resistance in ovarian cancer cells in vitro and in vivo. Cancer Biol Ther. 2010;10:788–95.
62. Eitan R, Kushnir M, Lithwick-Yanai G, David MB, Hoshen M, Glezerman M, et al. Tumor microRNA expression patterns associated with resistance to platinum based chemotherapy and survival in ovarian cancer patients. Gynecol Oncol. 2009;114:253–9.
63. Vecchione A, Belletti B, Lovat F, Volinia S, Chiappetta G, Giglio S, et al. A microRNA signature defines chemoresistance in ovarian cancer through modulation of angiogenesis. Proc Natl Acad Sci U S A. 2013;110:9845–50.
64. Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, et al. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008;68:10307–14.
65. Bagnoli M, De Cecco L, Granata A, Nicoletti R, Marchesi E, Alberti P, et al. Identification of a chrXq27.3 microRNA cluster associated with early relapse in advanced stage ovarian cancer patients. Oncotarget. 2011;2:1265–78.
66. Wilhelm-Benartzi CS, Koestler DC, Karagas MR, Flanagan JM, Christensen BC, Kelsey KT, et al. Review of processing and analysis methods for DNA methylation array data. Br J Cancer. 2013;109:1394–402.
67. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. REporting recommendations for tumor MARKer prognostic studies (REMARK). Nat Clin Pract Urol. 2005;2:416–22.
68. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004;429:457–63.
69. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002;20:2429–40.
70. Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood. 2007;109:52–7.
71. Steele N, Finn P, Brown R, Plumb JA. Combined inhibition of DNA methylation and histone acetylation enhances gene re-expression and drug sensitivity in vivo. Br J Cancer. 2009;100:758–63.
72. Plumb JA, Strathdee G, Sludden J, Kaye SB, Brown R. Reversal of drug resistance in human tumor xenografts by 2'-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. Cancer Res. 2000;60:6039–44.
73. Fang F, Balch C, Schilder J, Breen T, Zhang S, Shen C, et al. A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. Cancer. 2010;116:4043–53.
74. Bauman J, Verschraegen C, Belinsky S, Muller C, Rutledge T, Fekrazad M, et al. A phase I study of 5-azacytidine and erlotinib in advanced solid tumor malignancies. Cancer Chemother Pharmacol. 2012;69:547–54.
75. Appleton K, Mackay HJ, Judson I, Plumb JA, McCormick C, Strathdee G, et al. Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. J Clin Oncol. 2007;25:4603–9.
76. Glasspool R, Gore M, Rustin G, McNeish I, Wilson R, Pledge S, et al. Randomized phase II study of decitabine in combination with carboplatin compared with carboplatin alone in patients with recurrent advanced ovarian cancer. J Clin Oncol. 2009;15S:5562.
77. Glasspool RM, Brown R, Gore ME, Rustin GJ, McNeish IA, Wilson RH, et al. A randomised, phase II trial of the DNA-hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in combination with carboplatin vs carboplatin alone in patients with recurrent, partially platinum-sensitive ovarian cancer. Br J Cancer. 2014;110:1923–9.
78. Fu S, Hu W, Iyer R, Kavanagh JJ, Coleman RL, Levenback CF, et al. Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. Cancer. 2011;117:1661–9.
79. Matei D, Fang F, Shen C, Schilder J, Arnold A, Zeng Y, et al. Epigenetic resensitization to platinum in ovarian cancer. Cancer Res. 2012;72:2197–205.
80. Glasspool R, Gore M, Rustin G, McNeish I, Wilson R, Pledge S, et al. Randomized phase II study of decitabine in combination with carboplatin compared with carboplatin alone in patients with recurrent advanced ovarian cancer. J Clin Oncol. 2009;15S:5562.
81. Dizon DS, Blessing JA, Penson RT, Drake RD, Walker JL, Johnston CM, et al. A phase II evaluation of belinostat and carboplatin in the treatment of recurrent or persistent platinum-resistant ovarian, fallopian tube, or primary peritoneal carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2012;125:367–71.
82. Dizon DS, Damstrup L, Finkler NJ, Lassen U, Celano P, Glasspool R, et al. Phase II activity of belinostat (PXD-101), carboplatin, and paclitaxel in women with previously treated ovarian cancer. Int J Gynecol Cancer. 2012;22:979–86.
83. Mackay HJ, Hirte H, Colgan T, Covens A, MacAlpine K, Grenci P, et al. Phase II trial of the histone deacetylase inhibitor belinostat in women with platinum resistant epithelial ovarian cancer and micropapillary (LMP) ovarian tumours. Eur J Cancer. 2010;46:1573–9.
84. Zeller C, Brown R. Therapeutic modulation of epigenetic drivers of drug resistance in ovarian cancer. Ther Adv Med Oncol. 2010;2:319–29.
85. Graham JS, Kaye SB, Brown R. The promises and pitfalls of epigenetic therapies in solid tumours. Eur J Cancer. 2009;45:1129–36.
86. Staub J, Chien J, Pan Y, Qian X, Narita K, Aletti G, et al. Epigenetic silencing of HSulf-1 in ovarian cancer: implications in chemoresistance. Oncogene. 2007;26:4969–78.

87. Nicholson LJ, Smith PR, Hiller L, Szlosarek PW, Kimberley C, Sehouli J, et al. Epigenetic silencing of argininosuccinate synthetase confers resistance to platinum-induced cell death but collateral sensitivity to arginine auxotrophy in ovarian cancer. Int J Cancer. 2009;125:1454–63.
88. Su HY, Lai HC, Lin YW, Liu CY, Chen CK, Chou YC, et al. Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. Int J Cancer. 2010;127:555–67.
89. Bram EE, Stark M, Raz S, Assaraf YG. Chemotherapeutic drug-induced ABCG2 promoter demethylation as a novel mechanism of acquired multidrug resistance. Neoplasia. 2009;11:1359–70.
90. Iramaneerat K, Rattanatunyong P, Khemapech N, Triratanachat S, Mutirangura A. HERV-K hypomethylation in ovarian clear cell carcinoma is associated with a poor prognosis and platinum resistance. Int J Gynecol Cancer. 2011;21:51–7.
91. Syed N, Coley HM, Sehouli J, Koensgen D, Mustea A, Szlosarek P, et al. Polo-like kinase Plk2 is an epigenetic determinant of chemosensitivity and clinical outcomes in ovarian cancer. Cancer Res. 2011; 71:3317–27.
92. Ali MW, Cacan E, Liu Y, Pierce JY, Creasman WT, Murph MM, et al. Transcriptional suppression, DNA methylation, and histone deacetylation of the regulator of G-protein signaling 10 (RGS10) gene in ovarian cancer cells. PLoS One. 2013;8:e60185.
93. Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 2008;68:425–33.
94. Li Z, Hu S, Wang J, Cai J, Xiao L, Yu L, et al. MiR-27a modulates MDR1/P-glycoprotein expression by targeting HIPK2 in human ovarian cancer cells. Gynecol Oncol. 2010;119:125–30.
95. Ye G, Fu G, Cui S, Zhao S, Bernaudo S, Bai Y, et al. MicroRNA 376c enhances ovarian cancer cell survival by targeting activin receptor-like kinase 7: implications for chemoresistance. J Cell Sci. 2011;124(Pt 3):359–68.
96. Kong F, Sun C, Wang Z, Han L, Weng D, Lu Y, et al. miR-125b confers resistance of ovarian cancer cells to cisplatin by targeting pro-apoptotic Bcl-2 antagonist killer 1. J Huazhong Univ Sci Technolog Med Sci. 2011;31:543–9.
97. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, et al. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. Nat Med. 2011;17:1627–35.
98. Boyerinas B, Park SM, Murmann AE, Gwin K, Montag AG, Zillhardt M, et al. Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer. 2012;130:1787–97.
99. Cheng W, Liu T, Wan X, Gao Y, Wang H. MicroRNA-199a targets CD44 to suppress the tumorigenicity and multidrug resistance of ovarian cancer-initiating cells. FEBS J. 2012;279:2047–59.
100. Cittelly DM, Dimitrova I, Howe EN, Cochrane DR, Jean A, Spoelstra NS, et al. Restoration of miR-200c to ovarian cancer reduces tumor burden and increases sensitivity to paclitaxel. Mol Cancer Ther. 2012;11:2556–65.
101. Fu X, Tian J, Zhang L, Chen Y, Hao Q. Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells. FEBS Lett. 2012;586:1279–86.
102. Yang C, Cai J, Wang Q, Tang H, Cao J, Wu L, et al. Epigenetic silencing of miR-130b in ovarian cancer promotes the development of multidrug resistance by targeting colony-stimulating factor 1. Gynecol Oncol. 2012;124:325–34.
103. Yang L, Li N, Wang H, Jia X, Wang X, Luo J. Altered microRNA expression in cisplatin-resistant ovarian cancer cells and upregulation of miR-130a associated with MDR1/P-glycoprotein-mediated drug resistance. Oncol Rep. 2012;28:592–600.
104. Ziliak D, Gamazon ER, Lacroix B, Kyung Im H, Wen Y, Huang RS. Genetic variation that predicts platinum sensitivity reveals the role of miR-193b* in chemotherapeutic susceptibility. Mol Cancer Ther. 2012;11:2054–61.
105. Cai J, Yang C, Yang Q, Ding H, Jia J, Guo J, et al. Deregulation of let-7e in epithelial ovarian cancer promotes the development of resistance to cisplatin. Oncogenesis. 2013;2:e75.
106. Huh JH, Kim TH, Kim K, Song JA, Jung YJ, Jeong JY, et al. Dysregulation of miR-106a and miR-591 confers paclitaxel resistance to ovarian cancer. Br J Cancer. 2013;109:452–61.
107. Li X, Lu Y, Chen Y, Lu W, Xie X. MicroRNA profile of paclitaxel-resistant serous ovarian carcinoma based on formalin-fixed paraffin-embedded samples. BMC Cancer. 2013;13:216.
108. van Jaarsveld MT, Helleman J, Boersma AW, van Kuijk PF, van Ijcken WF, Despierre E, et al. miR-141 regulates KEAP1 and modulates cisplatin sensitivity in ovarian cancer cells. Oncogene. 2013;32:4284–93.
109. Prislei S, Martinelli E, Mariani M, Raspaglio G, Sieber S, Ferrandina G, et al. MiR-200c and HuR in ovarian cancer. BMC Cancer. 2013;13:72.
110. Rao YM, Shi HR, Ji M, Chen CH. MiR-106a targets Mcl-1 to suppress cisplatin resistance of ovarian cancer A2780 cells. J Huazhong Univ Sci Technolog Med Sci. 2013;33:567–72.
111. Zhang X, Huang L, Zhao Y, Tan W. Downregulation of miR-130a contributes to cisplatin resistance in ovarian cancer cells by targeting X-linked inhibitor of apoptosis (XIAP) directly. Acta Biochim Biophys Sin (Shanghai). 2013;45:995–1001.
112. Xiang Y, Ma N, Wang D, Zhang Y, Zhou J, Wu G, et al. MiR-152 and miR-185 co-contribute to ovarian cancer cells cisplatin sensitivity by targeting DNMT1 directly: a novel epigenetic therapy independent of decitabine. Oncogene. 2014;33:378–86.
113. Yu PN, Yan MD, Lai HC, Huang RL, Chou YC, Lin WC, et al. Downregulation of miR-29 contributes to cisplatin resistance of ovarian cancer cells. Int J Cancer. 2014;134:542–51.
114. Chan JK, Blansit K, Kiet T, Sherman A, Wong G, Earle C, et al. The inhibition of miR-21 promotes apoptosis and chemosensitivity in ovarian cancer. Gynecol Oncol. 2014;132:739–44.
115. Chen N, Chon HS, Xiong Y, Marchion DC, Judson PL, Hakam A, et al. Human cancer cell line microRNAs associated with in vitro sensitivity to paclitaxel. Oncol Rep. 2014;31:376–83.
116. Li B, Chen H, Wu N, Zhang WJ, Shang LX. Deregulation of miR-128 in ovarian cancer promotes cisplatin resistance. Int J Gynecol Cancer. 2014;24:1381–8.
117. Li H, Xu H, Shen H. microRNA-106a modulates cisplatin sensitivity by targeting PDCD4 in human ovarian cancer cells. Oncol Lett. 2014;7:183–8.
118. Zhu X, Li Y, Xie C, Yin X, Liu Y, Cao Y, et al. miR-145 sensitizes ovarian cancer cells to paclitaxel by targeting Sp1 and Cdk6. Int J Cancer. 2014;135:1286–96.
119. Zong C, Wang J, Shi TM. MicroRNA 130b enhances drug resistance in human ovarian cancer cells. Tumour Biol. 2014;35:12151–6.