Genetic and epigenetic heterogeneity of epithelial ovarian cancer and the clinical implications for molecular targeted therapy

Huimin Bai [a, b, #], **Dongyan Cao** [b, #], **Jiaxin Yang** [b], **Menghui Li** [a], **Zhenyu Zhang** [a, *], **Keng Shen** [b, *]

[a] *Department of Obstetrics and Gynecology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China*
[b] *Department of Obstetrics and Gynecology, Peking Union Medical College Hospital,*
Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

- Introduction
- Molecular portraits underlying TH of EOC

- Molecular targeted treatment
- Conclusions

Abstract

Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy, and tumoural heterogeneity (TH) has been blamed for treatment failure. The genomic and epigenomic atlas of EOC varies significantly with tumour histotype, grade, stage, sensitivity to chemotherapy and prognosis. Rapidly accumulating knowledge about the genetic and epigenetic events that control TH in EOC has facilitated the development of molecular-targeted therapy. Poly (ADP-ribose) polymerase (PARP) inhibitors, designed to target homologous recombination, are poised to change how breast cancer susceptibility gene (BRCA)-related ovarian cancer is treated. Epigenetic treatment regimens being tested in clinical or preclinical studies could provide promising novel treatment approaches and hope for improving patient survival.

Keywords: epithelial ovarian cancer ● EOC ● tumoural heterogeneity ● TH ● genetic and epigenetic alterations ● molecular targeted treatment

Introduction

Human epithelial ovarian cancer (EOC) is the most common cause of death from gynaecological malignancy [1]. The standard treatment for EOC involves cytoreductive surgery followed by chemotherapy consisting of platinum and taxol. For high-grade serous ovarian cancer (HGSOC), the most prevalent and aggressive form of EOC, relapse is nearly the norm due because of the development of resistance, although approximately 80% of patients initially respond to treatment [2]. Tumoural heterogeneity (TH) has been blamed for this treatment failure [3]. Gerlinger and Swanton [4] reported that genetic TH fosters the development of cancer drug resistance through Darwinian evolution, which points to a promising therapeutic target for preventing the evolution of more aggressive or resistant clones.

With the advent of next-generation sequencing in recent years, EOC has been found to consist of a complex set of diseases. Diverse genetic or epigenetic alterations that are of fundamental importance in tumorigenesis and progression have been identified in heterogeneous subsets of patients [5]. For example, breast cancer susceptibility gene (BRCA) mutations are most commonly associated with HGSOC [6]. Determining the molecular events that control this tumour trait might advance our understanding of tumorigenesis and facilitate individualized treatment strategies for this lethal disease.

Molecular portraits underlying TH of EOC

Underlying the hallmarks of cancers is genome instability, which can generate genetic diversity [7]. Genetic alterations can potentially

---

[#]These two authors contribute equally to this work.
*Correspondence to: Zhenyu ZHANG
E-mail: zhenyuzhang2000@163.com

Keng SHEN
E-mail: shenkeng@vip.sina.com; skpumch@126.com

upset the balance between proto-oncogenes and tumour suppressor genes, leading to tumorigenesis. The existence of extensive cytogenetic, genetic and epigenetic variations has been reported in EOC cell populations.

Numerical or structural chromosomal abnormalities are frequently observed in almost all human tumours [7]. Rearrangement of 19q has been identified in 61.6% of patients with ovarian cancer; such rearrangements have been significantly correlated with high-grade tumours, predicting shorter disease-free survival and worse overall survival (OS) [8] (Table 1). Underrepresentation of 11p and 13q and overrepresentation of 8q and 7p have been significantly correlated with undifferentiated ovarian carcinomas [9]. Underrepresentation of 12p and overrepresentation of 18p are frequently identified in well- and moderately differentiated ovarian tumours. Patients showing loss of D6S1581 are more likely to be resistant to platinum-based chemotherapy [10]. Gains of 14q32.33 have been associated with platinum resistance and reduced progression-free survival (PFS) and OS for patients with EOC [11]. Tumours exhibiting gain of 2p22-p25, 19p12-q13.1, and 20q12-q13 and loss of 5q14-q22 present a high risk of recurrence. The OS of patients is inversely correlated with the number of chromosomal alterations found in their tumours [12]. Gains at 5p are adversely associated with tumour recurrence [13], and gains at 1p and losses at 5q are associated with a significant decrease in recurrence. Loss at 6q24.2-26 is independently associated with a cluster of patients with HGSOC showing longer survival [14].

Gene copy number variations generally result in the abnormal expression of genes that are located within rearranged chromosomal regions. Nonrandom gains and deletions of DNA copy numbers and imbalances of alleles are frequently identified in ovarian tumours [15, 16]. Somatic copy number amplification is highly prevalent in high-grade ovarian cancer, whereas somatic mutational activation of oncogenes is a rare event, suggesting that the former is a common mechanism [17] of oncogene activation in this tumour type [15]. In addition, variations in gene copy number are specific to tumour histotypes, among which serous is the most prevalent, followed by endometrioid, clear cell and mucinous [17]. Mayr *et al.* [18] demonstrated that gains of FGF3/4 and CCNE1 occur in all serous carcinomas. Endometrioid carcinomas most frequently show gains of JUNB, KRAS2, MYCN, ESR and CCND2. Among serous borderline tumours, 80% exhibit amplification of FGFR1 and MDM2, and 75% show gains of PIK3CA (Table 1). By applying an *in silico* hypothesis-driven approach to multiple datasets, Huang *et al.* [17] found 76 cancer genes to be significantly altered in EOC, several of which may be potential copy number drivers, such as ERBB2 in mucinous tumours and TPM3 in endometrioid histotypes. In addition, KRAS was observed to be significantly amplified in serous tumours, although mutations are rare in such high-grade tumours. Copy number variations can also predict a patient's prognosis and response to treatment. Patients showing PIK3CA amplification generally respond well to treatment [19]. In contrast, amplification of 19q12 involving CCNE1 is the dominant structural variant associated with primary treatment failure of patients with HGSOC [20, 21]. Amplification of AKT2 is frequently identified in undifferentiated tumours and predicts a poor prognosis[22]. Ovarian cancer cells that either constitutively overexpress active Akt/AKT1 or exhibit AKT2 gene amplification are highly resistant to paclitaxel compared with cells with low AKT levels [23].

Overexpression of KLK6 [24], EGFR [25], LMX1B [26], BMP8B and ATP13A4 [27], because of gene amplification or high copy number gains, is associated with worse PFS and OS in patients with ovarian cancer. In contrast, an increased copy number of GAB2 is associated with improved PFS and OS and correlates with enhanced sensitivity to the dual PI3K/mTOR inhibitor PF-04691502 *in vitro* [27].

TP53 mutations are almost invariably present in HGSOC [15, 18, 20] (Table 1). The early loss of P53 function observed in sporadic cancers could create a permissive environment for the loss of BRCA1 or BRCA2 function (or other phenotypes of DNA repair deficiency), which would otherwise lead to apoptosis because of checkpoint activation [29]. Inactivation of BRCA1 and/or BRCA 2 is detected in 67% of patients with HGSOC, which is markedly higher than in the other histotypes of EOC [6]. However, only 7–9% of sporadic ovarian cancers exhibit BRCA1 [30] mutations leading to inactivation of BRCA1, while 4% exhibit BRCA2 mutations [31]. HGSOC tumours only form in animal models when all three of the BRCA, TP53 and PTEN genes are altered, which suggests a synergistic role of these genes in tumorigenesis [32]. Mutation in other genes, including FAT3, CSND3, NF1, CDK12, RB1 and GABRA6, are also frequently identified in HGSOC tumours [15]. Mutations in BRAF are restricted to serous borderline tumours, indicating that the majority of serous borderline tumours do not progress to serous carcinomas [33]. Activating KRAS mutations are more common in mucinous tumours than in all other histological types [17, 34], while no mucinous tumours have been found to harbour a BRAF mutation [34]. Loss or dysfunction of mismatch repair of gain-of-function PTEN [35] and PIK3CA [36] mutations is common in endometrioid and clear cell carcinoma, but not in serous or mucinous ovarian cancer [37]. Deletion of LRP1B in HGSOC is associated with acquired resistance to liposomal doxorubicin [38]. In addition to their histological implications, tumours with BRCA mutations are more likely to be platinum-sensitive and associated with longer PFS and OS [39, 40]. Reversion of germline BRCA1 or BRCA2 mutations in individual patients or loss of BRCA1 promoter methylation predicts resistance to platinum [20] and may also predict resistance to PARP ((poly (ADP-ribose) polymerase) inhibitors [41, 42].

Epigenetics is defined as heritable changes in gene expression that do not alter the DNA sequence itself. The mechanisms responsible for such changes include DNA methylation, histone modification, and microRNAs, which are related to post-transcriptional gene regulation. Epigenetic alterations are increasingly being implicated in the development and progression of ovarian cancer, and the gradual accumulation of epigenetic alterations has been associated with an advancing grade and stage of disease [43] (Table 2).

Methylation, which consists primarily of demethylation of oncogenes and hypermethylation of tumour suppressing genes, is frequently identified in ovarian cancer [44, 45]. Gene hypermethylation and satellite and global DNA hypomethylation in ovarian tumours are both independently associated with the degree of malignancy [46]. Satellite DNA hypomethylation is significantly more prevalent in advanced-stage and high-grade ovarian cancers and is an independent marker of poor prognosis [47]. In addition to repetitive elements and DNA satellites, hypomethylation of promoter CpG islands and gene overexpression have been reported in ovarian cancer. CpG islands are DNA sequences

containing CpG sites at an atypically high frequency [48] and are usually, but not exclusively, associated with gene promoters [49]. Demethylation of CpG islands in gene promoters generally allows active gene transcription to occur [50]. As a result of hypomethylation, re-expression of MCJ, SNCG, and BORIS and overexpression of CLDN4, MAL, BORIS [45] and TUBB3 [44] have been associated with chemoresistance in patients with EOC. As a result of promoter hypomethylation [51], HOXA10 is overexpressed in ovarian clear cell adenocarcinomas, but not in ovarian serous adenocarcinomas, normal ovarian epithelia or endometrial cysts [53]. In addition, this overexpression in ovarian clear cell adenocarcinomas [52, 53] is associated with poor survival [53] . DNA hypomethylation-mediated activation of the LINE-1 [54] and CT45 [55] genes is correlated with high-grade and advanced-stage EOC and associated with poorer PFS and OS.

Aberrant methylation of CpG islands in ovarian tumours is associated with silencing of genes involved in the control of the cell cycle, apoptosis and drug sensitivity as well as tumour suppressor genes [56]. Hypermethylation of the MLH1 gene, accompanied by loss of gene expression, and methylation of hMSH2 are correlated with a higher histological grade and lymph node metastasis of EOC [57]. In addition, methylation of the hMLH1 promoter has been identified in 56% of EOC patients with acquired resistance to platinum-based chemotherapy [58-60], predicting a high risk of relapse and poor OS [59]. The methylation rate of hMSH2 is significantly higher in endometrioid adenocarcinoma tissues compared with other histological types of the disease [57]. Epigenetic silencing of ARMCX2, COL1A1, MDK and MEST due to promoter hypermethylation at CpG sites has also been linked to the development of platinum-based resistance in ovarian cancer [60]. Methylation of DLEC1 is associated with recurrence of HGSOC, independent of tumour stage and suboptimal surgical debulking [61]. Chou *et al.* [62] reported that hypermethylation of the FBXO32 promoter is more commonly observed in advanced-stage ovarian tumours, and patients showing FBXO32 methylation exhibit significantly shorter PFS. Re-expression of FBXO32 was demonstrated to markedly reduce proliferation, increase apoptosis, and restore sensitivity to cisplatin in a platinum-resistant ovarian cancer cell line both *in vitro* and *in vivo*.

BRCA1 and BRCA2 germline mutations are present in the majority of patients with hereditary ovarian carcinoma [63], in contrast to the frequency of these mutations detected in unselected patients, which is only 15.3% [64]. The majority of ovarian cancers arise independently of mutations in the BRCA1/2 genes [65]. BRCA1/2 alterations of all kinds, including mutations, have been reported in up to 82% of ovarian tumours [31]. The term 'BRCAness' has been used to describe the phenotypic traits that some sporadic ovarian tumours share with tumours found in BRCA1/2 germline mutation carriers and reflects similar causative molecular abnormalities [66]. BRCAness appears to be the result of different epigenetic processes. Recent data suggest that hypermethylation of the BRCA1 promoter occurs in 10-15% of sporadic cases and is associated with the serous histotype [67, 68]. BRCA2 can also be down-regulated through silencing of its upstream regulator, FANCF, by promoter methylation [69, 70]. Although patients with BRCA1/2 mutations and low protein/mRNA expression of BRCA1 tend to show a favourable response to treatment[20] and a better outcome [40],

BRCA1 promoter methylation is significantly correlated with resistance to treatment [20] and a poorer prognosis [68] in patients with EOC. Thus, methylation is not functionally equivalent to a germline mutation in mediating chemotherapy sensitivity. While methylation of BRCA1 is common in sporadic ovarian cancer, it has not been reported in the hereditary form of the disease or in samples from women with germline BRCA1 mutations [71]. BRCA2 does not present a similar methylation profile in ovarian cancer [72].

DNA-associated histone proteins are subject to extensive modifications that mediate the assembly of transcriptionally permissive or repressive (*i.e.*, open or closed) chromatin. Chromatin modifiers regulate the expression of different sets of genes involved in tumorigenesis [73]. DNA methylation and histone deacetylation often coordinately inhibit gene transcription [74]. However, histone modification is an independent mechanism of epigenetic gene regulation under some conditions [75, 76]. H3K27m3 is a transcription-suppressive histone mark found in chromatin in association with EZH2, a component of the Polycomb (PcG) complex [77]. In ovarian cancer, decreased expression of H3K27me3 is significantly associated with high-grade and advanced-stage tumours, but not with the histological type [78], predicting resistance to chemotherapy [79] and a poor clinical outcome in ovarian cancer and other malignancies [78]. Removal of H3K27 methylation was shown to lead to re-expression of the RASSF1 tumour suppressor and resensitize drug-resistant ovarian cancer cells to cisplatin; this increased platinum access to DNA was likely due to relaxation of condensed chromatin [80]. Sirtuin-1 (SIRT1) is a nicotinamide adenine dinucleotide-dependent deacetylase and a class III histone deacetyltransferase. The proportion of SIRT1 expression is significantly higher in serous carcinoma compared with mucinous tumours. SIRT1 overexpression is more common in early-stage serous carcinomas and is correlated with longer OS compared with late-stage disease [81]. SIRT1 also facilitates the acquisition of drug resistance through its influence on the tumour microenvironment, function in DNA repair and promotion of cancer stem cell survival [82]. Thus, SIRT1 is being considered as a possible target for overcoming drug resistance in many malignancies.

Having been implicated in the initiation and progression of human cancers, microRNAs regulate processes such as cell growth, differentiation and apoptosis [83]. A variety of miRNAs are associated with tumour subtype, stage, grade, therapy resistance and prognosis in ovarian cancer [84] (Table 2). Up-regulation of miR-205 [85] and miR-200a [86] and down-regulation of miR-101 [87] are significantly associated with a high pathological grade and advanced stage of EOC in patients. In addition, patients with lymph node metastasis show significant elevation of miR-200c [86]. Reduced expression of miR-34b*/c [88], hsa-miR-200a, hsa-miR-34a and hsa-miR-449b [89] is frequently identified in advanced-stage tumours. Hsa-miR-378 [89] and let-7i [90] are up-regulated in patients who are sensitive to platinum; in contrast, miR-101, [87] miR-30c, miR-130a and miR-335 [91] are down-regulated in several resistant ovarian cancer cell lines, suggesting direct involvement in the development of chemoresistance. MiR-214 induces cell survival and cisplatin resistance through targeting the

© 2016 The Authors.   583

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

3′-UTR of the PTEN gene, which leads to reduced expression of PTEN and activation of the Akt pathway [92]. Down-regulation of miRNA-149 decreases the sensitivity of ovarian cancer cells to paclitaxel treatment by increasing MyD88 expression [93]. MiR-197 is significantly increased in Taxol-resistant ovarian cancer cells [94]. In addition, decreased expression of let-7i [90] and overexpression of miR-200a and miR-200c [86] are associated with shorter PFS, suggesting their potential for predicting relapse. Over-expression of miR-200, miR-141, miR-18a, miR-93 and miR-429 [95] is associated with improved OS, whereas high levels of hsa-miR-27a, [89] let-7b and miR-199a [95] are potentially correlated with a poor prognosis in patients with EOC.

Molecular targeted treatment

The rapid development of genetics and epigenetics has facilitated the study of the molecular mechanisms of TH in EOC. This knowledge has led to the introduction of novel treatments that are rationally designed to target specific molecular factors implicated in tumour growth (Table 3).

Dysfunction of BRCA1 and BRCA2 is associated with ovarian cancer tumorigenesis, due to an inability to repair DNA double-strand breaks (DSBs) [96]. The PARPs are a family of enzymes involved in base excision repair, a key pathway in the repair of DNA single-strand breaks (SSBs). PARP inhibition leads to the persistence of spontaneously occurring SSBs and subsequent formation of DSBs, as the SSBs stall and collapse replication forks. These DSBs cannot be repaired by the defective HR pathway in BRCA-mutated cells, resulting in cell death.

PARP inhibitors induce synthetic lethality in BRCA-deficient tissues. BRCA1/2-deficient cancers are now recognized as the target of a class of drugs known as PARP inhibitors. Deficiency of either PARP or BRCA alone has no impact, but deficiency in both leads to a lethal effect [97, 98]. Clinical investigation of the use of PARP inhibitors for the treatment of EOC evolved rapidly from the observations of single-agent activity conducted *in vitro* in BRCA-deficient cancer cells in 2005 to the initiation of multiple phase 3 studies in 2013. Ledermann *et al.* [99] retrospectively analysed the data from a randomized, double-blind, phase 2 study [100] and showed that patients with recurrent, platinum-sensitive serous ovarian cancer with a BRCA mutation exhibit the highest likelihood of benefiting from olaparib, the first human PARP inhibitor. Two phase III studies have been carried out to test olaparib *versus* placebo as maintenance therapy for both newly diagnosed and platinum-sensitive recurrent BRCA-associated ovarian cancer [101]. In December 2014, olaparib was approved for the treatment of patients with germline BRCA1/2-associated advanced ovarian cancer who have received three or more lines of chemotherapy. This approval represents the first 'personalized' therapy for ovarian cancer [102]. Other PARP inhibitors that have been tested or are currently being tested in clinical trials for ovarian cancer include veliparib, niraparib, rucaparib and BMN673 [101]. In addition to ovarian cancer, PARP inhibitors have shown encouraging in for other BRCA1/2 mutation-related cancers, such as breast cancer [103], endometrial cancer [104], prostate cancer [105] and pancreatic cancer [106]. Future and ongoing trials will identify the most effective role of these agents for use in human cancer treatment.

The signalling cascade involving PI3K, AKT and mTOR plays a key role in mediating cell proliferation and survival and is one of the pathways that is frequently affected in human cancer [107]. Various genetic alterations that activate PI3K/AKT/mTOR signalling have been identified in ovarian cancer [108]. In a previous study, we demonstrated that PI3K/AKT/mTOR pathway activation is associated with significantly higher migratory and invasive capacities in subpopulations of human ovarian cancer cell lines [109]. Thus, this pathway is regarded as an attractive candidate for therapeutic interventions against EOC, and inhibitors targeting different components of the pathway are in various stages of clinical development. Thus far, results have been published only for a phase I trial of an AKT inhibitor, perifosine [110], and a phase II trial of an mTORC1 inhibitor, temsirolimus [111]. Perifosine plus docetaxel appears to be effective in patients with mutational activation of the PI3K/AKT pathway [110]. A phase II clinical trial is currently being conducted to investigate the efficacy of perifosine as well as the association between PIK3CA status and the response to treatment in patients with recurrent gynaecological malignancies, including ovarian cancer. In a GOG phase II trial, [111] temsirolimus monotherapy showed modest activity in persistent or recurrent EOC and primary peritoneal cancer, and PFS was just below that required to warrant the inclusion of unselected patients in phase III studies. Based on these results, a phase II trial is currently being conducted specifically targeting ovarian clear cell carcinoma, which often exhibits PI3K/AKT/mTOR activation [108]. This trial is aimed at examining the use of temsirolimus in combination with carboplatin and paclitaxel, followed by temsirolimus consolidation, as a first-line therapy for patients with ovarian cancer, and its results appear promising.

Because genetic alterations are almost impossible to reverse, the potential reversibility of epigenetic mechanisms makes them more attractive candidates for the prevention and treatment of ovarian carcinoma [112]. There are two types of DNA methylation inhibitors (DNMTIs): nucleoside and non-nucleoside analogues [44]. Nucleoside analogues, such as cytarabine and decitabine, can inhibit methylation when they are integrated into DNA and block the release of DNA methyltransferases by forming a covalent complex with these enzymes [113]. Cytarabine has been reported to induce re-expression of hMLH1 and reverse drug resistance in human tumour xenografts through demethylation of the hMLH1 promoter [114]. Zebularine can also induce demethylation of hMLH1 and RASSF1A and resensitize drug-resistant cell lines to cisplatin [115]. The ability of azacitidine and decitabine to reverse platinum resistance in ovarian cancer patients has been preliminarily confirmed in two clinical trials [116, 117].

Inhibitors of histone deacetylation (HDACIs) represent another promising new class of anticancer agents. Among the currently available HDACIs, four have been tested in ovarian cancer, including vorinostat, romidepsin, valproate and PXD101. Vorinostat and romidepsin have both been approved by the FDA for the treatment of cutaneous T-cell lymphoma. Both agents, in combination with cytotoxic agents, have shown significant activity in inhibiting ovarian cancer cell growth in preclinical studies [118–120]. However, in a phase II study, vorinostat displayed minimal activity as a single agent for treating persistent or recurrent epithelial ovarian or primary peritoneal carci-

Table 1 Cytogenetic and genetic tumour heterogeneity in EOC

Molecular events	Heterogeneous clinicopathological characteristics				
	Histology	Grade	Response to CT	Relapse risk	Survival
Chromosomal abnormalities					
Rearrangement of 19q [8]	HGSOC			High	Adverse
Underrepresentation of 11p and 13q; overrepresentation of 8q and 7p [9]		High			
12p underrepresentation and 18p overrepresentation [9]		Low			
Loss of D6S1581 [10]			Resistant		
Gains of 14q32.33 [11]			Resistant	High	Adverse
Gains of 2p22p25, 19p12q13.1 and 20q12q13 and loss of 5q14q22 [12]				High	Adverse
Gain in 5p [13]				High	
Gain in 1p and loss in 5q [13]				Low	
Loss at 6q24.2-26 [14]					Favourable
Gene copy number variation					
Gains of FGF3/4 and CCNE1 [18]	Serous				
KRAS amplification [17]	HGSOC, rare in mucinous tumour				
Gain of JUNB, KRAS2, MYCN, ESR and CCND2 [18]; TPM3 amplification [17]	Endometrioid				
ERBB2 amplification [17]	Mucinous				
Amplification of FGFR1 and MDM2; gain of PIK3CA [18]		Borderline			
PIK3CA amplification [20]			Sensitive		
CCNE1 amplification [20, 21]; Akt/AKT1 overexpression [23]			Resistant		
AKT2 amplification		High [22]	Resistant [23]		Adverse [22]

Table 1. Continued

Molecular events	Heterogeneous clinicopathological characteristics				
	Histology	Grade	Response to CT	Relapse risk	Survival
Amplification of KLK6 [24], EGFR [25], LMX1B [26], BMP8B, and ATP13A4 [27]				High	Adverse
GAB2 amplification [27]			Sensitive	Low	Favourable
Somatic gene mutation					
TP53 [15, 20, 28]; FAT3, CSND3, NF1, CDK12, RB1, and GABRA6 [15]	HGSOC				
BRCA1/2	HGSOC [6]		Sensitive [39, 40]		Favourable [39, 40]
Reversions of germline BRCA1 or BRCA2 mutations or loss of BRCA1 promoter methylation [20, 41, 42]			Resistant		Adverse
BRAF	Not mucinous [34]; Serous Borderline tumours [33]				
KRAS [17, 34]	Mucinous	High			
PTEN loss [35]; PIK3CA mutation with gain of function [36]	Endometrioid and clear cell carcinoma				
LRP1B deletion [38]	HGSOC		Resistant		

CT: chemotherapy; HGSOC: high-grade serous ovarion cancer; EOC: epithelial ovarian cancer.

Table 2 Epigenetic tumour heterogeneity in EOC

Molecular events	Heterogeneous clinicopathological characteristics					
	Histology	Grade	Stage	Response to CT	Relapse risk	Survival
Hypomethylation						
Satellite DNA hypomethylation [47]		High	Advanced			Adverse
Re-expression of MCJ, SNCG, and BORIS [45]; overexpression of CLDN4, MAL, BORIS, and TUBB3 [44]				Resistant		
LINE-1 [54] and CT45 [55]		High	Advanced		High	Adverse
HOXA10 promoter hypomethylation	CCC [52, 53], rare in serous tumour [53]					Adverse [53]
Hypermethylation or methylation						
MLH1Hypermethylation [57]		High		Resistant		
hMLH1 promoter methylation				Resistant [58–60]	High [59]	Adverse [59]
hMSH2 [57]	Endometrioid	High				
DLEC1 methylation [60]	HGSOC		Advanced		High	
FBXO32 promoter hypermethylation or methylation [62]			Advanced	Resistant	High	
Promoter hypermethylation of ARMCX2, COL1A1, MDK, and MEST [60]				Resistant		
BRCA1 promoter hypermethylation	Serous [67, 68]			Resistant [20]		Adverse [68]
Histone modification						
H3-K27 m3 loss		High [78]	Advanced [78]	Resistant [79, 80]		Adverse [78]
Proportion of SIRT1 expression [79]	Serous					
SIRT1 overexpression [81]	Serous		Early			Favorable
MiRNAs						
Up-regulation of miR-205 [85]		High	Advanced			
Up-regulation of miR-200a [86]		High	Advanced		High	
Down-regulation of miR-101 [87]		High	Advanced	Resistant		

Table 2. Continued

Molecular events	Heterogeneous clinicopathological characteristics					
	Histology	Grade	Stage	Response to CT	Relapse risk	Survival
Reduced expression of miR-34b*/c [88], hsa-miR-200a, hsa-miR-34a, and hsa-miR-449b [89]			Advanced			
Up-regulation of Hsa-miR-378 [89]				Sensitive		
Reduced expression of miR-30c, miR-130a, miR-335 [91], and miRNA-149 [94]; overexpression of MiR-214 [92] and MiR-197 [94]				Resistant		
Overexpression of miR-200c [86]					High	
Reduced expression of let-7i [90]				Resistant	High	
Overexpression of miR-200, miR-141, miR-18a, miR-93, and miR-429 [95]						Favourable
Overexpression of hsa-miR-27a [89], let-7b, and miR-199a [95]						Adverse

Table 3 Molecular-targeted treatments for EOC

Drug	Condition	Treatment regimen	Trial phase
Targeting homologous recombination (PARP inhibitors)			
Olaparib [101, 102]	BRCA-associated ovarian cancer in both newly diagnosed and platinum-sensitive recurrent settings	Combined with post-platinum based CT	Phase III
Veliparib [101]	Recurrent HGSC (both germline BRCA and sporadic allowed)	Combined with Temozolomide	Phase II
Niraparib [101]	Recurrent platinum-sensitive ovarian cancer	Combined with post-platinum based CT	Phase III
Rucaparib [101]	Recurrent platinum-sensitive ovarian cancer	Combined with post-platinum based CT	Phase III
BMN673 [101]	Advanced or recurrent EOC	Single agent	Phase I
Targeting the PI3K/AKT/mTOR pathway			
Perifosine [110]	Recurrent EOC	Combined with docetaxel	Phase II
Temsirolimus [111]	Primary, persistent or recurrent EOC	Single agent	Phase II

Table 3. Continued

Drug	Condition	Treatment regimen	Trial phase
Targeting aberrant DNA methylation			
Cytarabine [114]	CT-resistant EOC	Single agent	Preclinical
Zebularine [115]	CT-resistant EOC	Combined with cisplatin	Preclinical
Azacitidine [116]	Platinum-resistant	Combined with platinum	Phase Ib-IIa
Decitabine [117]	Recurrent or platinum-resistant EOC	Combined with platinum	Phase I
Targeting histone modifications			
Vorinostat [121]	Persistent or recurrent EOC	Single agent	Phase II
Romidepsin [122]	Related data not available		Phase II
Valproate [123, 124]	Primary or resistant EOC	Single agent or combined with platinum	Preclinical
PXD101 [125]	CT-resistant EOC	Combined with platinum	Preclinical
Targeting miRNA dysregulation			
MiR-124 [129]	Advanced EOC	Single agent	Preclinical

noma, despite its acceptable tolerability [121]. A phase II trial examining the use of romidepsin for the treatment of ovarian cancer is ongoing [122]. Valproate exhibits direct HDACI activity, although the associated mechanisms of action remain unclear. Valproate is effective in sensitizing ovarian cancer cells to cisplatin and resensitizing cisplatin-resistant cells, both alone and in combination with other drugs [123, 124]. PXD101 can increase the acetylation of A-tubulin induced by docetaxel and the phosphorylation of H2AX induced by carboplatin. In addition, this drug can effectively reverse drug tolerance in both *in vitro* and *in vivo* models of ovarian cancer [125].

DNA methylation and histone modifications are intimately linked [74]. Hence, combining two classes of epigenetic drugs, DNMTIs and HDACIs, with conventional therapies may be a more effective approach in the clinic [126].

The dysregulation of miRNA expression in tumours makes miRNAs another potential therapeutic target, necessitating the specific identification of genes that are targets of miRNA regulation. The overexpression of miRNAs that act as oncogenes can be targeted for downregulation through the use of anti-miRNA oligonucleotides, miRNA masking, miRNA sponges or small molecule inhibitors. In contrast, restoring the activity of tumour suppressor miRNAs can inhibit proliferation and induce apoptosis of tumour cells, and miRNA mimics are applicable under these conditions [127]. Several clinical trials have been initiated to test the efficacy of miRNA-based therapeutics for the treatment of leukaemia, prostate cancer, and skin cancer [128] . As for ovarian cancer, this therapeutic approach is still at a preclinical stage to the best of our knowledge. Having identified miR-124 as a potential tumour suppressor that can functionally target the p27/myc/phospho-Rb protein signature, Seviour *et al.* [129] demonstrated that nanoparticle-mediated delivery of miR-124 can reduce tumour growth and sensitize cells to etoposide in a xenograft model. These findings present an exciting opportunity for the potential therapeutic use of miR-124 in combination with chemotherapy in patients with late-stage EOC.

Conclusions

Epithelial ovarian cancer is a heterogeneous disease. As discussed above, the genomic and epigenomic atlas of EOC varies significantly with tumour histotypes, grades and stages as well as with a patient's prognosis and sensitivity to chemotherapy. The rapidly increasing knowledge about the genetic and epigenetic events that control TH in EOC is facilitating the development of molecular targeted therapy. PARP inhibitors, which are designed to target HR, are poised to change how BRCA-related ovarian cancer is treated, representing the first 'personalized' therapy for ovarian cancer. Epigenetic treatment regimens being tested in preclinical or clinical studies are giving rise to optimism regarding the improvement of patient survival and may also provide promising novel treatment approaches.

Acknowledgements

© 2016 The Authors.

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

589

High Technology Research and Development Program of China (nos'. 2012AA02A507, 2014AA020606).

Disclosure

The authors have no conflict of interest to declare.

Author contribution

References

1. **Siegel R, Ma J, Zou Z, et al.** Cancer statistics, 2014. *CA Cancer J Clin*. 2014; 64: 9–29.
2. **Cooke SL, Brenton JD.** Evolution of platinum resistance in high-grade serous ovarian cancer. *Lancet Oncol*. 2011; 12: 1169–74.
3. **Campbell LL, Polyak K.** Breast tumor heterogeneity: cancer stem cells or clonal evolution? *Cell Cycle*. 2007; 6: 2332–8.
4. **Gerlinger M, Swanton C.** How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br J Cancer*. 2010; 103: 1139–43.
5. **Chao SY, Chiang JH, Huang AM, et al.** An integrative approach to identifying cancer chemoresistance-associated pathways. *BMC Med Genomics*. 2011; 4: 23.
6. **Mavaddat N, Barrowdale D, Andrulis IL, et al.** Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer Epidemiol Biomarkers Prev*. 2012; 21: 134–47.
7. **Sniegowski PD, Gerrish PJ, Johnson T, et al.** The evolution of mutation rates: separating causes from consequences. *BioEssays*. 2000; 22: 1057–66.
8. **Bayani J, Marrano P, Graham C, et al.** Genomic instability and copy-number heterogeneity of chromosome 19q, including the kallikrein locus, in ovarian carcinomas. *Mol Oncol*. 2011; 5: 48–60.
9. **Kiechle M, Jacobsen A, Schwarz-Boeger U, et al.** Comparative genomic hybridization detects genetic imbalances in primary ovarian carcinomas as correlated with grade of differentiation. *Cancer*. 2001; 91: 534–40.
10. **Makhija S, Sit A, Edwards R, et al.** Identification of genetic alterations related to chemoresistance in epithelial ovarian cancer. *Gynecol Oncol*. 2003; 90: 3–9.
11. **Despierre E, Moisse M, Yesilyurt B, et al.** Somatic copy number alterations predict response to platinum therapy in epithelial ovarian cancer. *Gynecol Oncol*. 2014; 135: 415–22.
12. **Hu J, Khanna V, Jones MW, et al.** Comparative study of primary and recurrent ovarian serous carcinomas: comparative genomic hybridization analysis with a potential application for prognosis. *Gynecol Oncol*. 2003; 89: 369–75.
13. **Bruchim I, Israeli O, Mahmud SM, et al.** Genetic alterations detected by comparative genomic hybridization and recurrence rate in epithelial ovarian carcinoma. *Cancer Genet Cytogenet*. 2009; 190: 66–70.
14. **Kamieniak MM, Rico D, Milne RL, et al.** Deletion at 6q24.2-26 predicts longer survival of high-grade serous epithelial ovarian cancer patients. *Mol Oncol*. 2015; 9: 422–36.
15. **Bell D, Berchuck A, Birrer M, Chien J, Cramer D, Dao F, et al.** Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011; 474: 609–15.
16. **Gorringe KL, Jacobs S, Thompson ER, et al.** High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. *Clin Cancer Res*. 2007; 13: 4731–9.
17. **Huang RY, Chen GB, Matsumura N, et al.** Histotype-specific copy-number alterations in ovarian cancer. *BMC Med Genomics*. 2012; 5: 47.
18. **Mayr D, Kanitz V, Anderegg B, et al.** Analysis of gene amplification and prognostic markers in ovarian cancer using comparative genomic hybridization for microarrays and immunohistochemical analysis for tissue microarrays. *Am J Clin Pathol*. 2006; 126: 101–9.
19. **Shayesteh L, Lu Y, Kuo WL, et al.** PIK3CA is implicated as an oncogene in ovarian cancer. *Nat Genet*. 1999; 21: 99–102.
20. **Patch AM, Christie EL, Etemadmoghadam D, et al.** Whole-genome characterization of chemoresistant ovarian cancer. *Nature*. 2015; 521: 489–94.
21. **Etemadmoghadam D, deFazio A, Beroukhim R, et al.** Integrated genome-wide DNA copy number and expression analysis identifies distinct mechanisms of primary chemoresistance in ovarian carcinomas. *Clin Cancer Res*. 2009; 15: 1417–27.
22. **Bellacosa A, de Feo D, Godwin AK, et al.** Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer*. 1995; 64: 280–5.
23. **Page C, Lin HJ, Jin Y, et al.** Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. *Anticancer Res*. 2000; 20: 407–16.
24. **Shan SJ, Scorilas A, Katsaros D, et al.** Transcriptional upregulation of human tissue kallikrein 6 in ovarian cancer: clinical and mechanistic aspects. *Br J Cancer*. 2007; 96: 362–72.
25. **Despierre E, Vergote I, Anderson R, et al.** Epidermal growth factor receptor (EGFR) pathway biomarkers in the randomized phase III trial of erlotinib *versus* observation in ovarian cancer patients with no evidence of disease progression after first-line platinum-based chemotherapy. *Target Oncol*. 2015; 10: 583–96.
26. **He L, Guo L, Vathipadiekal V, et al.** Identification of LMX1B as a novel oncogene in human ovarian cancer. *Oncogene*. 2014; 33: 4226–35.
27. **Davis SJ, Sheppard KE, Anglesio MS, et al.** Enhanced GAB2 expression is associated with improved survival in high-grade serous ovarian cancer and sensitivity to PI3K inhibition. *Mol Cancer Ther*. 2015; 14: 1495–503.
28. **Ahmed AA, Etemadmoghadam D, Temple J, et al.** Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. *J Pathol*. 2010; 221: 49–56.
29. **Venkitaraman AR.** Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*. 2002; 108: 171–82.
30. **Geisler JP, Hatterman-Zogg MA, Rathe JA, et al.** Frequency of BRCA1 dysfunction in ovarian cancer. *J Natl Cancer Inst*. 2002; 94: 61–7.
31. **Hilton JL, Geisler JP, Rathe JA, et al.** Inactivation of BRCA1 and BRCA2 in ovar-

ian cancer. *J Natl Cancer Inst.* 2002; 94: 1396–406.

32. **Perets R, Wyant GA, Muto KW, *et al.*** Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. *Cancer Cell.* 2013; 24: 751–65.

33. **Sieben NL, Macropoulos P, Roemen GM, *et al.*** In ovarian neoplasms, BRAF, but not KRAS, mutations are restricted to low-grade serous tumours. *J Pathol.* 2004; 202: 336–40.

34. **Gemignani ML, Schlaerth AC, Bogomolniy F, *et al.*** Role of KRAS and BRAF gene mutations in mucinous ovarian carcinoma. *Gynecol Oncol.* 2003; 90: 378–81.

35. **Obata K, Morland SJ, Watson RH, *et al.*** Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. *Cancer Res.* 1998; 58: 2095–7.

36. **Kuo KT, Mao TL, Jones S, *et al.*** Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. *Am J Pathol.* 2009; 174: 1597–601.

37. **Verhaak RG, Tamayo P, Yang JY, *et al.*** Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. *J Clin Invest.* 2013; 123: 517–25.

38. **Cowin PA, George J, Fereday S, *et al.*** LRP1B deletion in high-grade serous ovarian cancers is associated with acquired chemotherapy resistance to liposomal doxorubicin. *Cancer Res.* 2012; 72: 4060–73.

39. **Vencken PM, Kriege M, Hoogwerf D, *et al.*** Chemosensitivity and outcome of BRCA1- and BRCA2-associated ovarian cancer patients after first-line chemotherapy compared with sporadic ovarian cancer patients. *Ann Oncol.* 2011; 22: 1346–52.

40. **Sun C, Li N, Ding D, *et al.*** The role of BRCA status on the prognosis of patients with epithelial ovarian cancer: a systematic review of the literature with a meta-analysis. *PLoS ONE.* 2014; 9: e95285.

41. **Norquist B, Wurz KA, Pennil CC, *et al.*** Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *J Clin Oncol.* 2011; 29: 3008–15.

42. **Sakai W, Swisher EM, Karlan BY, *et al.*** Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature.* 2008; 451: 1116–20.

43. **Balch C, Fang F, Matei DE, *et al.*** Minireview: epigenetic changes in ovarian cancer. *Endocrinology.* 2009; 150: 4003–11.

44. **Koukoura O, Spandidos DA, Daponte A, *et al.*** DNA methylation profiles in ovarian cancer: implication in diagnosis and therapy. *Mol Med Rep.* 2014; 10: 3–9.

45. **Matei DE, Nephew KP.** Epigenetic therapies for chemosensitization of epithelial ovarian cancer. *Gynecol Oncol.* 2010; 116: 195–201.

46. **Ehrlich M, Woods CB, Yu MC, *et al.*** Quantitative analysis of associations between DNA hypermethylation, hypomethylation, and DNMT RNA levels in ovarian tumors. *Oncogene.* 2006; 25: 2636–45.

47. **Widschwendter M, Jiang G, Woods C, *et al.*** DNA hypomethylation and ovarian cancer biology. *Cancer Res.* 2004; 64: 4472–80.

48. **Illingworth RS, Bird AP.** CpG islands–'a rough guide'. *FEBS Lett.* 2009; 583: 1713–20.

49. **Bernstein BE, Meissner A, Lander ES.** The mammalian epigenome. *Cell.* 2007; 128: 669–81.

50. **Weber M, Schubeler D.** Genomic patterns of DNA methylation: targets and function of an epigenetic mark. *Curr Opin Cell Biol.* 2007; 19: 273–80.

51. **Cheng W, Jiang Y, Liu C, *et al.*** Identification of aberrant promoter hypomethylation of HOXA10 in ovarian cancer. *J Cancer Res Clin Oncol.* 2010; 136: 1221–7.

52. **Cheng W, Liu J, Yoshida H, *et al.*** Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. *Nat Med.* 2005; 11: 531–7.

53. **Li B, Jin H, Yu Y, *et al.*** HOXA10 is overexpressed in human ovarian clear cell adenocarcinoma and correlates with poor survival. *Int J Gynecol Cancer.* 2009; 19: 1347–52.

54. **Pattamadilok J, Huapai N, Rattanatanyong P, *et al.*** LINE-1 hypomethylation level as a potential prognostic factor for epithelial ovarian cancer. *Int J Gynecol Cancer.* 2008; 18: 711–7.

55. **Zhang W, Barger CJ, Link PA, *et al.*** DNA hypomethylation-mediated activation of Cancer/Testis Antigen 45 (CT45) genes is associated with disease progression and reduced survival in epithelial ovarian cancer. *Epigenetics.* 2015; 10: 736–48.

56. **Barton CA, Hacker NF, Clark SJ, *et al.*** DNA methylation changes in ovarian cancer: implications for early diagnosis, prognosis and treatment. *Gynecol Oncol.* 2008; 109: 129–39.

57. **Zhang H, Zhang S, Cui J, *et al.*** Expression and promoter methylation status of mis-

match repair gene hMLH1 and hMSH2 in epithelial ovarian cancer. *Aust N Z J Obstet Gynaecol.* 2008; 48: 505–9.

58. **Watanabe Y, Ueda H, Etoh T, *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: 1449–52.

59. **Gifford G, Paul J, Vasey PA, *et al.*** The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients. *Clin Cancer Res.* 2004; 10: 4420–6.

60. **Zeller C, Dai W, Steele NL, *et al.*** Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. *Oncogene.* 2012; 31: 4567–76.

61. **Montavon C, Gloss BS, Warton K, *et al.*** Prognostic and diagnostic significance of DNA methylation patterns in high grade serous ovarian cancer. *Gynecol Oncol.* 2012; 124: 582–8.

62. **Chou JL, Su HY, Chen LY, *et al.*** Promoter hypermethylation of FBXO32, a novel TGF-beta/SMAD4 target gene and tumor suppressor, is associated with poor prognosis in human ovarian cancer. *Lab Invest.* 2010; 90: 414–25.

63. **Frank TS, Manley SA, Olopade OI, *et al.*** Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. *J Clin Oncol.* 1998; 16: 2417–25.

64. **Pal T, Permuth-Wey J, Betts JA, *et al.*** BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer.* 2005; 104: 2807–16.

65. **Weberpals JI, Koti M, Squire JA.** Targeting genetic and epigenetic alterations in the treatment of serous ovarian cancer. *Cancer Genet.* 2011; 204: 525–35.

66. **Rigakos G, Razis E.** BRCAness: finding the Achilles heel in ovarian cancer. *Oncologist.* 2012; 17: 956–62.

67. **Yang HJ, Liu VW, Wang Y, *et al.*** Differential DNA methylation profiles in gynecological cancers and correlation with clinicopathological data. *BMC Cancer.* 2006; 6: 212.

68. **Wiley A, Katsaros D, Chen H, *et al.*** Aberrant promoter methylation of multiple genes in malignant ovarian tumors and in ovarian tumors with low malignant potential. *Cancer.* 2006; 107: 299–308.

69. **Taniguchi T, Tischkowitz M, Ameziane N, *et al.*** Disruption of the Fanconi anemia-

BRCA pathway in cisplatin-sensitive ovarian tumors. *Nat Med*. 2003; 9: 568–74.

70. **Lim SL, Smith P, Syed N, et al.** Promoter hypermethylation of FANCF and outcome in advanced ovarian cancer. *Br J Cancer*. 2008; 98: 1452–6.

71. **Bol GM, Suijkerbuijk KP, Bart J, et al.** Methylation profiles of hereditary and sporadic ovarian cancer. *Histopathology*. 2010; 57: 363–70.

72. **Kontorovich T, Cohen Y, Nir U, et al.** Promoter methylation patterns of ATM, ATR, BRCA1, BRCA2 and p53 as putative cancer risk modifiers in Jewish BRCA1/BRCA2 mutation carriers. *Breast Cancer Res Treat*. 2009; 116: 195–200.

73. **Ozdag H, Teschendorff AE, Ahmed AA, et al.** Differential expression of selected histone modifier genes in human solid cancers. *BMC Genom*. 2006; 7: 90.

74. **Jones PA, Baylin SB.** The epigenomics of cancer. *Cell*. 2007; 128: 683–92.

75. **Caslini C, Capo-chichi CD, Roland IH, et al.** Histone modifications silence the GATA transcription factor genes in ovarian cancer. *Oncogene*. 2006; 25: 5446–61.

76. **Chan MW, Huang YW, Hartman-Frey C, et al.** Aberrant transforming growth factor beta1 signaling and SMAD4 nuclear translocation confer epigenetic repression of ADAM19 in ovarian cancer. *Neoplasia*. 2008; 10: 908–19.

77. **Muller J, Hart CM, Francis NJ, et al.** Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell*. 2002; 111: 197–208.

78. **Wei Y, Xia W, Zhang Z, et al.** Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol Carcinog*. 2008; 47: 701–6.

79. **Chapman-Rothe N, Curry E, Zeller C, et al.** Chromatin H3K27me3/H3K4me3 histone marks define gene sets in high-grade serous ovarian cancer that distinguish malignant, tumour-sustaining and chemoresistant ovarian tumour cells. *Oncogene*. 2013; 32: 4586–92.

80. **Abbosh PH, Montgomery JS, Starkey JA, 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.

81. **Jang KY, Kim KS, Hwang SH, et al.** Expression and prognostic significance of SIRT1 in ovarian epithelial tumours. *Pathology*. 2009; 41: 366–71.

82. **Wang Z, Chen W.** Emerging roles of SIRT1 in cancer drug resistance. *Genes Cancer*. 2013; 4: 82–90.

83. **Iorio MV, Croce CM.** MicroRNAs in cancer: small molecules with a huge impact. *J Clin Oncol*. 2009; 27: 5848–56.

84. **Van Jaarsveld MT, Helleman J, Berns EM, et al.** MicroRNAs in ovarian cancer biology and therapy resistance. *Int J Biochem Cell Biol*. 2010; 42: 1282–90.

85. **Niu K, Shen W, Zhang Y, et al.** MiR-205 promotes motility of ovarian cancer cells *via* targeting ZEB1. *Gene*. 2015; 574: 330–336.

86. **Zuberi M, Mir R, Das J, et al.** Expression of serum miR-200a, miR-200b, and miR-200c as candidate biomarkers in epithelial ovarian cancer and their association with clinicopathological features. *Clin Transl Oncol*. 2015; 17: 779–87.

87. **Liu L, Guo J, Yu L, et al.** miR-101 regulates expression of EZH2 and contributes to progression of and cisplatin resistance in epithelial ovarian cancer. *Tumour Biol*. 2014; 35: 12619–26.

88. **Corney DC, Hwang CI, Matoso A, et al.** Frequent downregulation of miR-34 family in human ovarian cancers. *Clin Cancer Res*. 2010; 16: 1119–28.

89. **Eitan R, Kushnir M, Lithwick-Yanai G, 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.

90. **Yang N, Kaur S, Volinia S, 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.

91. **Sorrentino A, Liu CG, Addario A, et al.** Role of microRNAs in drug-resistant ovarian cancer cells. *Gynecol Oncol*. 2008; 111: 478–86.

92. **Yang H, Kong W, He L, 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.

93. **Ibrahim FF, Jamal R, Syafruddin SE, et al.** MicroRNA-200c and microRNA-31 regulate proliferation, colony formation, migration and invasion in serous ovarian cancer. *J Ovarian Res*. 2015; 8: 56.

94. **Zou D, Wang D, Li R, et al.** MiR-197 induces Taxol resistance in human ovarian cancer cells by regulating NLK. *Tumour Biol*. 2015; 36: 6725–6732.

95. **Nam EJ, Yoon H, Kim SW, et al.** MicroRNA expression profiles in serous ovarian carcinoma. *Clin Cancer Res*. 2008; 14: 2690–5.

96. **Yoshida K, Miki Y.** Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci*. 2004; 95: 866–71.

97. **Safra T, Borgato L, Nicoletto MO, et al.** BRCA mutation status and determinant of outcome in women with recurrent epithelial ovarian cancer treated with pegylated liposomal doxorubicin. *Mol Cancer Ther*. 2011; 10: 2000–7.

98. **Murai J, Huang SY, Das BB, et al.** Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. *Cancer Res*. 2012; 72: 5588–99.

99. **Ledermann J, Harter P, Gourley C, et al.** Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol*. 2014; 15: 852–61.

100. **Ledermann J, Harter P, Gourley C, et al.** Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med*. 2012; 366: 1382–92.

101. **Liu JF, Konstantinopoulos PA, Matulonis UA.** PARP inhibitors in ovarian cancer: current status and future promise. *Gynecol Oncol*. 2014; 133: 362–9.

102. **Walsh CS.** Two decades beyond BRCA1/2: homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecol Oncol*. 2015; 137: 343–50.

103. **Tutt A, Robson M, Garber JE, et al.** Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet*. 2010; 376: 235–44.

104. **Forster MD, Dedes KJ, Sandhu S, et al.** Treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. *Nat Rev Clin Oncol*. 2011; 8: 302–6.

105. **Sandhu SK, Schelman WR, Wilding G, et al.** The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. *Lancet Oncol*. 2013; 14: 882–92.

106. **Lowery MA, Kelsen DP, Stadler ZK, et al.** An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions. *Oncologist*. 2011; 16: 1397–402.

107. **Chalhoub N, Baker SJ.** PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol.* 2009; 4: 127–50.

108. **Mabuchi S, Kuroda H, Takahashi R, *et al.*** The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. *Gynecol Oncol.* 2015; 137: 173–9.

109. **Bai H, Li H, Li W, *et al.*** The PI3K/AKT/mTOR pathway is a potential predictor of distinct invasive and migratory capacities in human ovarian cancer cell lines. *Oncotarget.* 2015; 6: 25520–32.

110. **Fu S, Hennessy BT, Ng CS, *et al.*** Perifosine plus docetaxel in patients with platinum and taxane resistant or refractory high-grade epithelial ovarian cancer. *Gynecol Oncol.* 2012; 126: 47–53.

111. **Behbakht K, Sill MW, Darcy KM, *et al.*** Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a Gynecologic Oncology Group study. *Gynecol Oncol.* 2011; 123: 19–26.

112. **Ushijima T, Asada K.** Aberrant DNA methylation in contrast with mutations. *Cancer Sci.* 2010; 101: 300–5.

113. **Santi DV, Norment A, Garrett CE.** Covalent bond formation between a DNA-cytosine methyltransferase and DNA containing 5-azacytosine. *Proc Natl Acad Sci USA.* 1984; 81: 6993–7.

114. **Plumb JA, Strathdee G, Sludden J, *et al.*** 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.

115. **Balch C, Yan P, Craft T, *et al.*** Antimitogenic and chemosensitizing effects of the methylation inhibitor zebularine in ovarian cancer. *Mol Cancer Ther.* 2005; 4: 1505–14.

116. **Fu S, Hu W, Iyer R, *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.

117. **Fang F, Balch C, Schilder J, *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.

118. **Dietrich CS 3rd, Greenberg VL, DeSimone CP, *et al.*** Suberoylanilide hydroxamic acid (SAHA) potentiates paclitaxel-induced apoptosis in ovarian cancer cell lines. *Gynecol Oncol.* 2010; 116: 126–30.

119. **Chen MY, Liao WS, Lu Z, *et al.*** Decitabine and suberoylanilide hydroxamic acid (SAHA) inhibit growth of ovarian cancer cell lines and xenografts while inducing expression of imprinted tumor suppressor genes, apoptosis, G2/M arrest, and autophagy. *Cancer.* 2011; 117: 4424–38.

120. **Wilson AJ, Lalani AS, Wass E, *et al.*** Romidepsin (FK228) combined with cisplatin stimulates DNA damage-induced cell death in ovarian cancer. *Gynecol Oncol.* 2012; 127: 579–86.

121. **Modesitt SC, Sill M, Hoffman JS, *et al.*** A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol.* 2008; 109: 182–6.

122. **Marsh DJ, Shah JS, Cole AJ.** Histones and their modifications in ovarian cancer - drivers of disease and therapeutic targets. *Front Oncol.* 2014; 4: 144.

123. **Lin CT, Lai HC, Lee HY, *et al.*** Valproic acid resensitizes cisplatin-resistant ovarian cancer cells. *Cancer Sci.* 2008; 99: 1218–26.

124. **Shan Z, Feng-Nian R, Jie G, *et al.*** Effects of valproic acid on proliferation, apoptosis, angiogenesis and metastasis of ovarian cancer *in vitro* and *in vivo*. *Asian Pac J Cancer Prev.* 2012; 13: 3977–82.

125. **Qian X, LaRochelle WJ, Ara G, *et al.*** Activity of PXD101, a histone deacetylase inhibitor, in preclinical ovarian cancer studies. *Mol Cancer Ther.* 2006; 5: 2086–95.

126. **Asadollahi R, Hyde CA, Zhong XY.** Epigenetics of ovarian cancer: from the lab to the clinic. *Gynecol Oncol.* 2010; 118: 81–7.

127. **Maradeo ME, Cairns P.** Translational application of epigenetic alterations: ovarian cancer as a model. *FEBS Lett.* 2011; 585: 2112–20.

128. **Wahid F, Shehzad A, Khan T, *et al.*** MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta.* 2010; 1803: 1231–43.

129. **Seviour EG, Sehgal V, Lu Y, *et al.*** Functional proteomics identifies miRNAs to target a p27/Myc/phospho-Rb signature in breast and ovarian cancer. *Oncogene.* 2016; 35: 691–701.

© 2016 The Authors.

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

593