Therapeutic modulation of epigenetic drivers of drug resistance in ovarian cancer

Ther Adv Med Oncol
(2010) 2(5) 319–329
DOI: 10.1177/1758834010375759

Constanze Zeller and Robert Brown

***Abstract*:** Epigenetic changes in tumours are associated not only with cancer development and progression, but also with resistance to chemotherapy. Aberrant DNA methylation at CpG islands and associated epigenetic silencing are observed during the acquisition of drug resistance. However, it remains unclear whether all of the observed changes are drivers of drug resistance, causally associated with response of tumours to chemotherapy, or are passenger events representing chance DNA methylation changes. Systematic approaches that link DNA methylation and expression with chemosensitivity will be required to identify key drivers. Such drivers will be important prognostic or predicitive biomarkers, both to existing chemotherapies, but also to epigenetic therapies used to modulate drug resistance.

***Keywords*:** CpG islands, DNA methylation, drug resistance, epigenetics, histones, ovarian cancer, therapies

Introduction

Although there have been substantial advances in current chemotherapeutic strategies, clinical drug resistance remains a major obstacle to successful cancer treatment and is still a limiting factor in patient survival [Broxterman *et al.* 2009]. This problem is particularly obvious in the treatment of ovarian cancer [Agarwal and Kaye, 2003]. Although around 80% of ovarian cancer patients initially respond to primary chemotherapy, the majority will relapse and eventually develop resistance to currently available treatment options. Conventionally, standard treatment consists of platinum-based drugs (carboplatin/cisplatin) which are either given as a single agent or in combination with the taxane, paclitaxel [Greenlee *et al.* 2001]. The chemotherapeutic activity of platinum drugs is mainly based on their ability to form DNA adducts and the 1,2-d (GpG) intrastrand crosslink has been particularly implicated. Here, the platinum coordinates the N7 atoms of adjacent guanosines in the DNA strand, which is believed to be poorly repaired in cells, with persistence of the lesion ultimately interfering with replication and transcription leading to cell death [Wang and Lippard, 2005; Kartalou and Essigmann, 2001]. Ovarian cancer is defined clinically as being 'platinum resistant' if the tumour recurrence occurs less than 6 months after completion of platinum-based first-line chemotherapy. An improved response to second-line treatment is seen if the tumour recurs later than 6 months following the end of first-line treatment and chances for a 'platinum-sensitive' response are greatly enhanced if the relapse occurs after 12 months.

CpG island methylation and drug resistance

A number of genetic alterations have been suggested to underlie the phenomenon of drug resistance, such as alterations in genes involved in DNA repair, drug uptake, apoptosis and cell cycle checkpoints [Broxterman *et al.* 2009; Luqmani, 2005]. However, in recent years it has become increasingly apparent that aberrant epigenetic mechanisms may also play a crucial role in drug resistance (for a summary of epigenetic mechanisms, see Figure 1). DNA methylation is one of the major epigenetic mechanisms controlling gene expression and cell differentiation [Bird, 1996]. Regions of high CpG dinucleotide density, called CpG islands, are frequently located in the promoters of house-keeping genes and are usually free of methylation in normal cells [Bird, 2002]. In cancer, CpG islands can become hypermethylated, contributing for example to silencing of tumour suppressor genes. This has been demonstrated for cancer susceptibility genes such as *BRCA1* [Press *et al.* 2008].

Correspondence to:
Robert Brown, PhD
Department of Oncology, IRDB, Hammersmith Hospital Campus, Imperial College London, Du Cane Road, London, W12 0NN, UK
b.brown@imperial.ac.uk

Constanze Zeller, PhD
Department of Oncology, IRDB, Hammersmith Hospital Campus, Imperial College London, Du Cane Road, London W12 0NN, UK

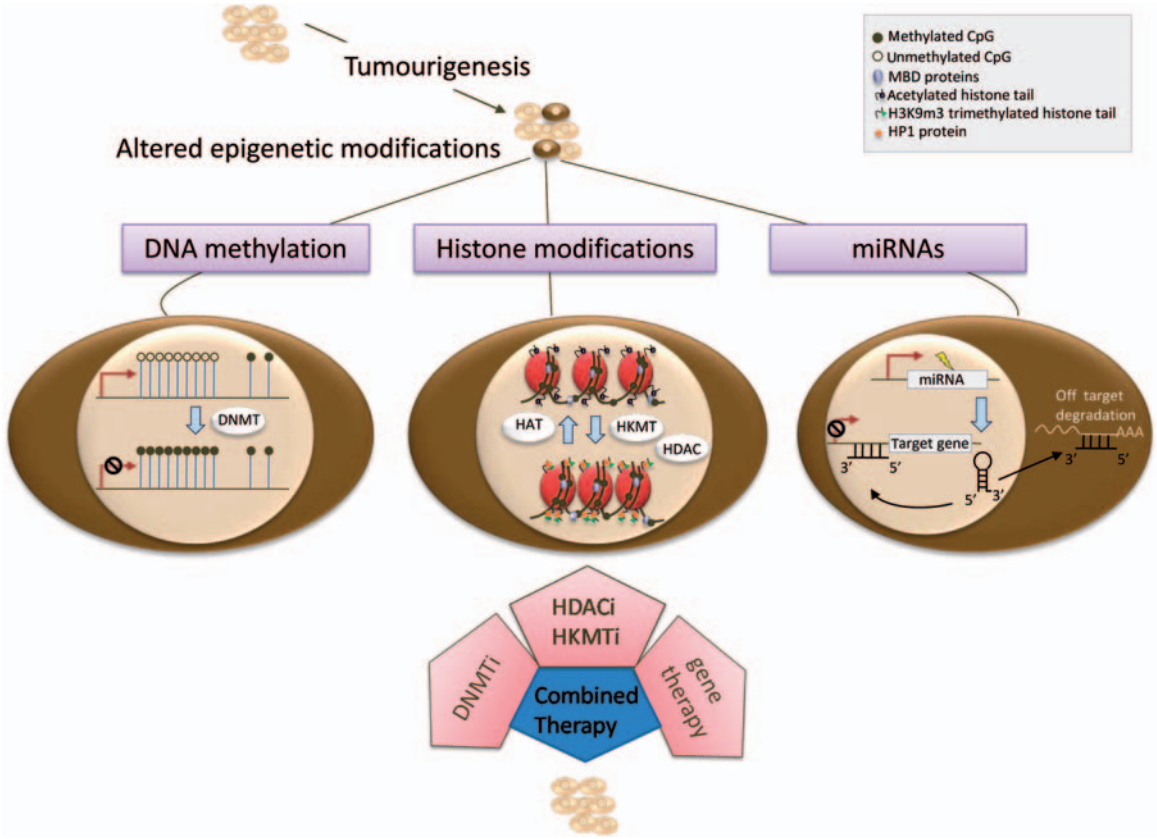

Figure 1. Possible targets for epigenetic therapy approaches in chemoresistant cells. Aberrant DNA hypermethylation (dark circles) at CpG islands can lead to transcriptional inactivation of genes and is frequently observed in tumours. Inhibition of the enzymes catalysing DNA methylation (DNMTs) leads to a genome-wide decrease of DNA methylation levels, thereby potentially re-activating vital anticancer genes. Each histone modification is established via specific enzymes (HDACs and HKMTs) catalysing the addition or removal of certain marks. Targeting aberrant hypoacetylation via HDACi can result in the re-expression of former transcriptionally incompetent chromatin. Similarly, the inhibition of certain aberrantly active histone methyltransferases (HKMTs) prevents methyl marks which may lead to repression of genes, as seen following the binding of HP1 (orange circles) to H3K9me3. Next to the two major epigenetic mechanisms of DNA methylation and histone modification, aberrant expression of miRNAs (small non-protein-coding RNAs of 21–23 nucleotides) has been correlated with tumourigenesis. Here, miRNAs potentially act on two pathways, the transcriptional silencing mechanism and the translational silencing mechanism. If in either case the miRNAs promiscuously bind sequences, for instance via mutation, the former specific gene regulation is out of control, possibly contributing to tumourigenesis and chemoresistance. In ovarian cancer, for example, downregulation of the miRNA *let-7i* increases resistance to cisplatin and is associated with shorter progression-free survival time of patients with late-stage ovarian cancer [Yang *et al.* 2008]. Although not yet available, gene therapy may be a tool to either re-establish lost endogenous miRNA expression or silence aberrant miRNA expression thereby complementing existing epigenetic therapies.

Furthermore, methylation of the CpG island linked to *BRCA1* is associated with good response to platinum-based chemotherapy in ovarian cancer [Teodoridis *et al.* 2005]. Mutation of the *BRCA1*/*BRCA2* genes is also associated with good response to platinum-based chemotherapy [Cass *et al.* 2003]. Conversely, reversion mutations at *BRCA2* have been shown to be associated with platinum resistance [Edwards *et al.* 2008]. It remains to be established whether reversal of *BRCA* methylation is a mechanism of acquired resistance. In ovarian cancer cell lines, methylation of the *FANCF* gene has been observed to be associated with increased sensitivity to cisplatin [Taniguchi *et al.* 2003]. FANCF is crucial for the activation of a DNA repair complex containing BRCA1 and BRCA2. Treatment with the demethylating agent 2′-deoxy-5-azacytidine led to demethylation of the *FANCF* gene and reduced sensitivity towards

cisplatin in these cell line models [Taniguchi *et al.* 2003]; however, again, the relevance of *FANCF* methylation to clinical outcome following chemotherapy is still to be established.

In contrast to *BRCA* and *FANCF* where epigenetic or genetic inactivation of the gene is associated with drug sensitivity, inactivation of genes involved in engaging an apoptotic response would lead to drug resistance [Teodoridis *et al.* 2005]. For instance, methylation of the DNA mismatch repair gene *MLH1* and transcriptional silencing occurs in cisplatin-resistant ovarian cell line models. MLH1 has been shown to be necessary for engagement of a variety of downstream cellular responses to alkylating agents and cisplatin-induced DNA damage [Papouli *et al.* 2004; Stojic *et al.* 2004]. It has been argued that, since mismatch repair (MMR) proteins can recognize and bind to certain types of damage in DNA, this is necessary for MMR-dependent engagement of DNA damage responses such as activation of p53, p73 and other downstream apoptosis signalling pathways [Stojic *et al.* 2004; Shimodaira *et al.* 2003; Duckett *et al.* 1999]. Hence, loss of MLH1 expression may lead to reduced engagement of apoptosis either due to reduced cycles of futile repair [Karran and Hampson, 1996], reduced stalling (or increased bypass) of lesions in DNA during DNA replication [Moreland *et al.* 1999] or direct signalling of cell death pathways [Yoshioka *et al.* 2006]. Acquired methylation at the *MLH1* locus has been observed following platinum-based chemotherapy in ovarian cancer, which has been shown to be associated with poor patient survival [Gifford *et al.* 2004].

Increased methylation of *DAPK* might also have an implication in chemoresistance. Hypermethylation of *DAPK* occurs frequently in tumours such as colon and breast tumours [Yamaguchi *et al.* 2003; Lehmann *et al.* 2002] and is an indicator of poor clinical outcome in lung cancer patients [Tang *et al.* 2000]. The calcium/calmodulin-regulated serine/threonin kinase DAPK has a pro-apoptotic role which is mediated via interferon (IFN)-γ, Fas and tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [Tang *et al.* 2004; Cohen *et al.* 1999; Inbal *et al.* 1997]. Highlighting the potential role of DAPK in chemoresistance, it has been shown that TRAIL-resistant lung cancer cell lines can be resensitized by using DNMT inhibitors leading to demethylation and re-expression of *DAPK* [Tang *et al.* 2004]. Following onto this, in gastric cancer patients the promoter methylation of *DAPK* was also shown to correlate with shorter progression-free survival and lower response rates to chemotherapeutic treatment with 5-fluorouracil [Kato *et al.* 2008]. Consequently, the methylation of members of the apoptotic and anti-apoptotic machinery such as *DAPK* have the potential to influence apoptosis and hence chemosensitivity.

Epigenetic drug resistance drivers

It has been proposed that drug resistance is a polygenic phenotype caused by the cumulative effect of multiple drug resistance or sensitivity associated genes, rather than phenotypic effects on drug sensitivity of a few loci [Glasspool *et al.* 2006]. Indeed large numbers of gene expression changes are observed in tumours and during acquired drug resistance [Konstantinopoulos *et al.* 2008], as well as extensive changes in DNA methylation, which then may lead to the drug-resistant phenotype. One of the current challenges is to identify the key changes which drive a cell towards a drug-resistant phenotype among the vast majority of epigenetic changes caused, for instance, by global demethylation or localized hypermethylation of CpG islands [Ehrlich, 2006]. Many of these global changes could have occurred by chance either as part of a methylator phenotype or simply as random silencing events [Issa, 2004]. In other words: how do we discriminate a 'driver' which, in analogy to tumourigenesis, has a functional effect that provides the cell with a selective advantage from a 'passenger' that has no functional impact but was incidentally acquired [Greenman *et al.* 2007]?

For driver mutations of cancer it has been estimated that only 8–16% of all reported mutations within protein kinase genes represent true drivers [Carter *et al.* 2009; Greenman *et al.* 2007]. Moreover, a substantial number of these changes are only present at a low frequency [Wood *et al.* 2007]. By analogy, it might be only a very specific subset of epigenetic changes transforming a sensitive tumour cell into a resistant one. Few large-scale studies have addressed the issue of acquired drug resistance in ovarian cancer on an epigenome-wide basis. Dai *et al.* [2008] analysed methylation changes associated with acquired cisplatin resistance in isogenic ovarian cancer cell lines and showed that hypermethylation of CpG islands is prevalent during the acquisition of drug resistance. To take this further, Li and colleagues

Table 1. Classes and names of DNA methylation inhibitors and histone deacetylase inhibitors, their targets and clinical status.

Type of epigenetic therapy	Class of compound	Compound	Target	Development stage
DNA methylation inhibitor	Nucleoside analogue	5′-azacytidine (Vidaza)	DNMTs	Approved for MDS, CMML and AML
		5-aza-2′-deoxycytidine (Decitabine/ Dacogen)	DNMTs	Approved for MDS
		Zebularine	DNMTs	Preclinical
	Non-nucleoside analogue	RG 108	DNMT1	Preclinical
		Procaine	DNMT1	Preclinical
		Procainamide	DNMT1	Preclinical
		Hydralazine	DNMT1	Phase II
	Antisense	MG98	DNMT1	Phase II
Histone deacetylase inhibitor	Hydroxamate	Suberoylanilide hydroxamic acid (SAHA, Vorinostat)	Class I, II	Approved for advanced CTCL
		PXD101	Class I, II	Phase II
		LAQ824, LBH589	Class I, II	Phase I
		Trichostatin A	Class I, II	Preclinical
		Oxamflatin, Scriptaid, SBHA	N/A	Preclinical
		Pyroxamide	Class I, unknown effect on class II	Phase I
		SK-7041, SK-7068	HDAC1, 2	Preclinical
		Tubacin	HDAC6	Preclinical
	Aliphatic acid	Valproic acid (VPA)	Class I, II	Phase II, III
		Phenylbutyrate	Class I, II	Phase I, II
		Savicol	N/A	Phase I, II
		AN-9 (prodrug)	N/A	Phase I, II
		Baceca	Class I	Phase I, II
	Benzamide	MS-275 (SNDX-275)	HDAC1, 2, 3 and slightly 8	Phase I, II
		MGCD0103	HDAC1, 2, 3, 11	Phase I, II
	Cyclic tetrapeptide	Depsipeptide (FK228)	Class I	Phase I, II
		Trapoxin A	Class I, II	Preclinical
		Apicidin	HDAC1, 3	Preclinical
		CHAPs	Class I	Preclinical

AML, acute myeloid leukaemia; CHAP, cyclic hydroxamic-acid-containing peptide; CMML, chronic myelomonocytic leukaemia; CTCL, cutaneous T-cell lymphoma; DNMT, DNA methyltransferase; DNMTi, DNMT inhibitor; HDAC, histone deacetylase; HDACi, HDAC inhibitor; MDS, myelodysplastic syndrome; N/A, not available; SBHA, suberic bishydroxamic acid. Adapted from Bolden *et al.* [2006], Yoo and Jones [2006] and Xu *et al.* [2007].

[2009] combined methylation with expression data and also identified extensive hypermethylation associated with epigenetic repression of loci implicated in cell adhesion as well as hypomethylation associated with activation of genes involved in the PI3K/AKT, transforming growth factor (TGF)-beta and cell cycle progression pathways. However, to pinpoint the epigenetic changes driving chemoresistance more precisely, it will be necessary to systematically investigate combined methylation, gene expression and resensitization in order to identify key drivers of the resistance phenotype. As an example, *MLH1* epigenetic silencing is selected for during platinum treatment of ovarian tumour cell lines and re-expression of *MLH1* either by demethylation or gene re-introduction resensitizes ovarian tumour cells to subsequent chemotherapeutic treatment [Plumb *et al.* 2000] and might, therefore, represent one of the key genes driving chemoresistance in the cell line models examined. However, this gives no indication of how many other genes may be key drivers of resistance, either in this model or in tumours in general. The actual proportion of epigenetically altered genes driving chemoresistance is still elusive. For future studies, systematic approaches will be required confirming a substantial effect of these methylation changes in terms of their ability to reverse drug resistance. If a small subset of genes drives drug resistance, this would be a valuable set of biomarkers for stratifying patients who may benefit from epigenetic approaches to resensitize tumours to chemotherapy.

Epigenetic modulators of drug resistance

It is widely recognized that a wide variety of epigenetic changes are prevalent in cancer [Jones and Baylin, 2007]. Importantly, unlike genetic mutations, epigenetic alterations are reversible and, therefore, the executing enzymes provide attractive drug targets for new therapies. Growing understanding of the mechanisms and enzymes governing epigenetic regulation has resulted in a variety of new drugs and possible drug targets for cancer treatment. These epigenetic therapies have the potential to re-activate or silence aberrantly regulated genes, thereby reversing many aspects of cancer phenotypes, including drug resistance. Examples of epigenetic therapies undergoing preclinical and clinical evaluation or registered for use in certain cancers are shown in Table 1.

DNA methylation inhibitors

So far, DNA methylation inhibitors (DNMTi) represent the most widely studied class of epigenetic therapeutic agents. The US Food and Drug Administration (FDA) approved 5-azacytidine (Vidaza) and Decitabine (5-aza-2′-deoxycytidine, Dacogen), cytidine ribose and deoxyribose nucleoside analogues, respectively [Issa, 2007]. Both drugs exert their effect by becoming incorporated into DNA, and inhibiting the DNA methyltransferase (DNMT) by forming covalent adducts with the enzyme, leading to its sequestration and cellular depletion. As a result of reduced DNMT activity during subsequent cell division DNA methylation is increasingly diminished. Consequently, cytosine methylation is reduced in newly replicated DNA, but not in the DNA of resting or non-dividing cells. In addition, Decitabine has been shown to induce depletion of DNMT1 through proteosomal degradation [Ghoshal *et al.* 2005]. In cell line models, these nucleoside DNA methylation inhibitors proved to effect methylation and to re-activate epigenetically silenced tumour suppressor genes [Mund *et al.* 2006]. Importantly, Decitabine induced re-expression of genes known to mediate drug response, for example *MLH1,* resulting in enhanced chemosensitivity to cytotoxic drugs [Plumb *et al.* 2000].

Vidaza and Decitabine have been clinically tested and have shown substantial therapeutic potential in haematological cancers such as leukaemias [Fenaux *et al.* 2009; Stewart *et al.* 2009; Issa *et al.* 2005; Issa *et al.* 2004]. A randomized phase III trial with myelodysplastic syndrome (MDS) patients supported the potency of 5-azacytidine to improve overall survival time of patients when compared with other therapies [Fenaux *et al.* 2009]. However, the effectiveness of DNMTi in solid tumours has been limited so far [Graham *et al.* 2009]. Several reasons may account for this, including their short half-life in plasma combined with the relatively low proliferation rate of solid tumours cells, limiting the amount of aza-nucleosides becoming incorporated into DNA. To overcome this problem, treatments with prolonged exposures to demethylating agents at lower doses may be necessary, in order to reverse methylation and restore gene activity rather than triggering cytotoxicity as seen with higher doses of DNMTi [Issa *et al.* 2004].

Furthermore, recent clinical trials have aimed to optimize currently available treatments by combining those with DNMTis to enhance susceptibility to conventional chemotherapeutic agents. For example, Decitabine has been studied as a modulator of resistance in combination with carboplatin in solid tumours in a phase I clinical trial [Appleton *et al.* 2007]. Encouragingly, demethylation rates were reported in peripheral blood cells as indicated by *MAGE1A* promoter methylation levels. Following on from the initial success, a randomized phase II trial was started in patients with recurrent ovarian cancer that had progressed within 6–12 months following the first round of platinum therapy [Glasspool *et al.* 2009]. However, the study was closed after a planned interim analysis due to poor feasibility and lack of efficacy of the combination. One possibility for the lack of efficacy seen in the phase II trial may be due to reduced dose intensity of the carboplatin and/or due to the additional myelosuppression associated with the Decitabine treatment. In order for these agents to progress within the solid tumour setting, it will be important to develop agents that are less myelosuppressive in combination with an altered schedule. For example, studies in haematological malignancies have used a 1 h infusion of Decitabine over 5–10 days. Regarding ovarian cancer, studies are currently underway combining platinum chemotherapy with similar schedules [Matei and Nephew, 2010]. Another feasible explanation may be that the demethylation of certain genes leads to an adverse effect counteracting the carboplatin sensitizing effect of demethylation. Again highlighting the importance of patient selection based on their tumour methylation profile for treatments and future studies.

More recently, much attention has been focused on developing small molecule inhibitors of DNMTs. RG 108 is one example of such a non-nucleoside compound which inhibits DNA methylation by directly blocking the active site of the enzyme [Brueckner *et al.* 2005]. RG 108 has been shown to restore the activity of epigenetically silenced tumour suppressor genes such as $p16^{Ink4}$ in human colon cancer cell lines, notably without the drug-induced toxicity usually observed with traditional DNMTi [Stresemann *et al.* 2006; Brueckner *et al.* 2005]. However, further improvement of the drug is highly desirable in terms of its cell uptake and efficacy [Brueckner *et al.* 2007]. The potential of non-nucleoside inhibitors to more specifically inhibit DNMTs, without causing non-specific DNA damage associated with nucleoside DNMT inhibitors, suggests that this class of compounds may have potential to be less-toxic therapies. However, clinical studies are needed to reveal more about their clinical applicability.

Histone modulators

Histone deacetylase inhibitors. The second intensively studied class of drugs targeting epigenetic silencing mechanisms are the histone deacetylase inhibitors (HDACis). Histone deacetylases (HDACs) can regulate the chromatin conformation through the removal of acetyl groups from the lysine residue of histone tails. Inhibiting these enzymes promotes accumulation of the acetylated form of histone proteins, ultimately leading to less-condensed packaging of genes in chromatin which may lead to the re-expression of silenced tumour suppressor genes. However, HDACis also target various other proteins, including key molecules regulating tumour cell growth, which may be responsible for the observed induction of different phenotypes in transformed cells such as proliferation arrest, differentiation and apoptosis [Egger *et al.* 2004]. Although the actual mechanism of growth inhibition might not necessarily be due to acetylation of histones *per se*, the fact that normal cells are usually not affected by HDACi-induced cell death at low concentrations supports their suitability as specific anticancer agents [Warrener *et al.* 2003]. Currently, a variety of up to 15 different HDACis are under investigation in phase I–III clinical trials [Marks and Xu, 2009].

The only HDACi approved by the FDA is Vorinostat (SAHA), a hydroxide acid derivative, registered for treatment of cutaneous T-cell lymphoma (since 2006). Vorinostat has shown proven efficacy in the treatment of haematologic malignancies [Garcia-Manero *et al.* 2008; Duvic *et al.* 2007; Olsen *et al.* 2007]. It has also been tested as a single agent in clinical phase I/II trials in various solid tumours including head and neck, breast and thyroid cancer. However, response rates suggested that the activity of this HDACi is low in solid tumour types [Batty *et al.* 2009]. Recently, Vorinostat has been shown to enhance the efficacy of the cytotoxic agents carboplatin and paclitaxel in patients with advanced non-small cell lung cancer in the setting of a clinical phase II trial [Ramalingam *et al.* 2010]. However, due to increased toxicity optimization

of the schedule seems to be required for this combination. The synergy of HDACi and conventional chemotherapeutic agents might be a promising route for the treatment of solid tumours.

Despite the initial encouraging results, monotherapy with DNMTis is limited due to the observed toxicity and the eventual re-methylation of genes [Plumb *et al.* 2004]. Now, there is growing interest in combined epigenetic therapies involving DNMTis and HDACis. For example, the DNMTi treatment can act synergistically with an HDACi in restoring gene expression [Cameron *et al.* 1999]. It has been proposed that DNA hypermethylation can lead to compact nucleosomes resistant to acetylation, thereby dominating silencing. A sequential administration of DNMTi prior to the HDACi administration appears to be important for sufficient efficacy in the treatment of solid tumours. Preclinical studies and early clinical trials are exploring the combination of epigenetic remodelling agents [Griffiths and Gore, 2008]. For example, the sequential treatment with Decitabine and the hydroxamate HDACi, PXD101 (Belinostat), has been tested in a cisplatin-resistant human ovarian tumour xenograft model [Steele *et al.* 2009; Plumb *et al.* 2004]. Combined treatment resulted in a marked increase in expression of epigenetically silenced *MLH1* and *MAGE1A* compared with treatment with Decitabine alone supporting the idea that combinatorial epigenetic therapy might improve sensitivity to chemotherapeutic agents. A recent clinical phase I/II trial combining the short fatty acid Valproic acid (VPA) (HDACi) with Decitabine was done in patients with leukaemia [Garcia-Manero *et al.* 2006]. Here, it emerged that this combination led to a transient reversal of epigenetic marks such as methylation of the *p15* promoter and deacetylation of histone H3 and H4. However, neither the level of DNA demethylation nor the level of acetylation could be correlated to clinical response.

Histone methyltransferase inhibitors. The discovery of histone methyltransferases (HKMT) has opened up another promising avenue to target aberrant epigenetic mechanisms [Lachner *et al.* 2003]. HKMTs are chromatin-modifying enzymes which establish methyl marks on the lysine of histone tail proteins. Depending on the position of the methyl mark the histone code can be read from binding proteins which then lead to transcriptional activation or repression of genes. Deregulation of HKMT activity has been linked to tumour development, especially high levels of the H3K27 methyltransferase EZH2 (polycomb group protein, enhancer of zeste homologue) have recently been observed in a variety of tumours including prostate, breast and melanoma and are associated with poor prognosis [Yu *et al.* 2007; Bachmann *et al.* 2006; Varambally *et al.* 2002]. Subsequent efforts to find appropriate inhibitors for EZH2 led to the discovery of 3-deazaneplanocin A (DZNep). DZNep is an S-adenosylhomocysteine hydrolase inhibitor capable of depleting EZH2 and other components of the Polycomb Repressive Complex 2 (PRC2) from cells *in vitro* and induces apoptosis in breast cancer cells [Tan *et al.* 2007]. However, subsequent studies showed that DZNep is not specific to EZH2 but is generally inhibiting methyltransferase activity thereby affecting global histone methylation levels [Miranda *et al.* 2009]. This is most likely due to its indirect mechanism of inhibition which blocks S-adenosyl methionine (SAM)-dependent methyltransferases through byproduct inhibition.

Nevertheless, DZNep is being explored to improve the epigenetic effect of HDACi at an early preclinical stage. Results seem encouraging, for example, treatment of an acute myeloid leukaemia cell line with combined DZNep and the HDACi panobinostat highly increased rates of apoptotic cell death in comparison with treatment with either drug alone [Fiskus *et al.* 2009]. However, there is a need for the development of novel, more selective inhibitors of HKMT which is supported by studies performed in glioblastoma multiform, an aggressive form of malignant glioma. Here, it was demonstrated that pharmacologic disruption of EZH2 by DZNep strongly impaired glioblastoma multiforme cancer stem cell self-renewal *in vitro* and tumour-initiating capacity *in vivo* due to repression of *c-myc*, a proto-oncogene mediating cell growth [Suva *et al.* 2009]. Therefore, the development of novel inhibitors with greater biological specificity is highly sought and may provide valuable tools for more targeted therapies.

Also in light of combined epigenetic therapy the current efforts to develop compounds targeting more distinct classes of epigenetic enzymes should greatly enhance their efficacy. The pleiotropic effects observed with anticancer agents

already in the clinic can often be a drawback and limit their value in therapy. More selectively acting compounds should aid in overcoming toxic effects currently seen. Combination studies indicate that sequential administration of drugs against different classes of enzymes might also increase efficacy of treatment. Although still at an early stage, future inhibitors of HKMT may represent a new class of compounds which could offer more selectivity compared with the currently available options.

Conclusions

It is becoming increasingly clear that epigenetic changes occurring during the acquisition of drug resistance are substantial and complex and might even outnumber genetic alterations. There are clear examples of the potential of epigenetic alterations at specific loci affecting drug resistance. However, in order to successfully target abnormal epigenetic changes in the clinic, it will be necessary to comprehensively identify the key events driving this process. Consequently, a therapy specifically targeting epigenetically deregulated genes could help in overcoming resistance. Longitudinal studies monitoring tumours acquiring resistance during the course of chemotherapy could provide an opportunity to map genome-wide epigenetic changes which may allow the correlation to acquisition of drug resistance. However, the magnitude of changes and heterogeneity among tumours will make such analyses challenging. Here, it will be crucial to perform epigenetic profiling of an appropriately sized panel of tumours pre- and postchemotherapy in order to achieve sufficient power for statistical analysis. In addition, those large-scale studies will also greatly expand the repertoire of available biomarkers which are vital for stratification of patients and for monitoring efficacy of existing epigenetic therapies.

Current efforts to isolate and characterize so-called cancer sustaining (stem) cells may yield new insights into genetic and epigenetic mechanisms governing chemoresistance [Ferrandina *et al.* 2008; Zhang *et al.* 2008; Agarwal and Kaye, 2003]. One of the features of such tumour sustaining cells is their inherent resistance to a number of chemotherapeutics, making them a suitable model for drug-resistance studies. Cancer sustaining cells have stem-cell-like properties in the way that they have the capacity to recapitulate the original tumour and promote recurrence.

Although successfully used in haematological malignancies DNMTi and HDACi work on a multitude of targets. The concern is that the genome-wide re-expression of aberrantly and normally regulated genes could lead to conflicting effects concerning response to chemotherapy. Therefore, the development of more targeted (epigenetic) therapies might be a prerequisite of successfully preventing drug resistance. The idea of reversing the malignant epigenetic marks of a small subset of genes driving drug resistance in order to sensitize tumours to chemotherapy will require novel strategies based on our increased understanding of the complexities of epigenetic regulation.

Funding

This research received funding from Cancer Research UK.

Conflict of interest statement

The authors declare they have no conflicts of interest.

References

Agarwal, R. and Kaye, S.B. (2003) Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer* 3: 502–516.

Appleton, K., Mackay, H.J., Judson, I., Plumb, J.A., McCormick, C., Strathdee, G. *et al.* (2007) Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. *J Clin Oncol* 25: 4603–4609.

Bachmann, I.M., Halvorsen, O.J., Collett, K., Stefansson, I.M., Straume, O., Haukaas, S.A. *et al.* (2006) EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J Clin Oncol* 24: 268–273.

Batty, N., Malouf, G.G. and Issa, J.P. (2009) Histone deacetylase inhibitors as anti-neoplastic agents. *Cancer Lett* 280: 192–200.

Bird, A. (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* 16: 6–21.

Bird, A.P. (1996) The relationship of DNA methylation to cancer. *Cancer Surv* 28: 87–101.

Bolden, J.E., Peart, M.J. and Johnstone, R.W. (2006) Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 5: 769–784.

Broxterman, H.J., Gotink, K.J. and Verheul, H.M. (2009) Understanding the causes of multidrug resistance in cancer: a comparison of doxorubicin and sunitinib. *Drug Resist Updat* 12: 114–126.

Brueckner, B., Garcia Boy, R., Siedlecki, P., Musch, T., Kliem, H.C., Zielenkiewicz, P. *et al.* (2005) Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res* 65: 6305–6311.

Brueckner, B., Kuck, D. and Lyko, F. (2007) DNA methyltransferase inhibitors for cancer therapy. *Cancer J* 13: 17–22.

Cameron, E.E., Bachman, K.E., Myohanen, S., Herman, J.G. and Baylin, S.B. (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet* 21: 103–107.

Carter, H., Chen, S., Isik, L., Tyekucheva, S., Velculescu, V.E., Kinzler, K.W. *et al.* (2009) Cancer-specific high-throughput annotation of somatic mutations: computational prediction of driver missense mutations. *Cancer Res* 69: 6660–6667.

Cass, I., Baldwin, R.L., Varkey, T., Moslehi, R., Narod, S.A. and Karlan, B.Y. (2003) Improved survival in women with BRCA-associated ovarian carcinoma. *Cancer* 97: 2187–2195.

Cohen, O., Inbal, B., Kissil, J.L., Raveh, T., Berissi, H., Spivak-Kroizaman, T. *et al.* (1999) DAP-kinase participates in TNF-alpha- and Fas-induced apoptosis and its function requires the death domain. *J Cell Biol* 146: 141–148.

Dai, W., Teodoridis, J.M., Graham, J., Zeller, C., Huang, T.H., Yan, P. *et al.* (2008) Methylation Linear Discriminant Analysis (MLDA) for identifying differentially methylated CpG islands. *BMC Bioinformatics* 9: 337.

Duckett, D.R., Bronstein, S.M., Taya, Y. and Modrich, P. (1999) hMutSalpha and MutLalpha dependent phosphorylation of p53 in response to DNA methylator damage. *Proc Natl Acad Sci USA* 96: 12384–12388.

Duvic, M., Talpur, R., Ni, X., Zhang, C., Hazarika, P., Kelly, C. *et al.* (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* 109: 31–39.

Edwards, S.L., Brough, R., Lord, C.J., Natrajan, R., Vatcheva, R., Levine, D.A. *et al.* (2008) Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 451: 1111–1115.

Egger, G., Liang, G., Aparicio, A. and Jones, P.A. (2004) Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429: 457–463.

Ehrlich, M. (2006) Cancer-linked DNA hypomethylation and its relationship to hypermethylation. *Curr Top Microbiol Immunol* 310: 251–274.

Fenaux, P., Mufti, G.J., Hellstrom-Lindberg, E., Santini, V., Finelli, C., Giagounidis, A. *et al.* (2009) Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 10: 223–232.

Ferrandina, G., Bonanno, G., Pierelli, L., Perillo, A., Procoli, A., Mariotti, A. *et al.* (2008) Expression of CD133-1 and CD133-2 in ovarian cancer. *Int J Gynecol Cancer* 18: 506–514.

Fiskus, W., Wang, Y., Sreekumar, A., Buckley, K.M., Shi, H., Jillella, A. *et al.* (2009) Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. *Blood* 114: 2733–2743.

Garcia-Manero, G., Kantarjian, H.M., Sanchez-Gonzalez, B., Yang, H., Rosner, G., Verstovsek, S. *et al.* (2006) Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. *Blood* 108: 3271–3279.

Garcia-Manero, G., Yang, H., Bueso-Ramos, C., Ferrajoli, A., Cortes, J., Wierda, W.G. *et al.* (2008) Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. *Blood* 111: 1060–1066.

Ghoshal, K., Datta, J., Majumder, S., Bai, S., Kutay, H., Motiwala, T. *et al.* (2005) 5-Aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. *Mol Cell Biol* 25: 4727–4741.

Gifford, G., Paul, J., Vasey, P.A., Kaye, S.B. and Brown, R. (2004) The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients. *Clin Cancer Res* 10: 4420–4426.

Glasspool, R.M., Gore, M., Rustin, G., McNeish, I., Wilson, R., Pledge, S. *et al.* (2009) Randomized phase II study of decitabine in combination with carboplatin compared with carboplatin alone in patients with recurrent advanced ovarian cancer. *J Clin Oncol* 27: 15s.

Glasspool, R.M., Teodoridis, J.M. and Brown, R. (2006) Epigenetics as a mechanism driving polygenic clinical drug resistance. *Br J Cancer* 94: 1087–1092.

Graham, J.S., Kaye, S.B. and Brown, R. (2009) The promises and pitfalls of epigenetic therapies in solid tumours. *Eur J Cancer* 45: 1129–1136.

Greenlee, R.T., Hill-Harmon, M.B., Murray, T. and Thun, M. (2001) Cancer statistics, 2001. *CA Cancer J Clin* 51: 15–36.

Greenman, C., Stephens, P., Smith, R., Dalgliesh, G.L., Hunter, C., Bignell, G. *et al.* (2007) Patterns of somatic mutation in human cancer genomes. *Nature* 446: 153–158.

Griffiths, E.A. and Gore, S.D. (2008) DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. *Semin Hematol* 45: 23–30.

Inbal, B., Cohen, O., Polak-Charcon, S., Kopolovic, J., Vadai, E., Eisenbach, L. *et al.* (1997) DAP kinase links the control of apoptosis to metastasis. *Nature* 390: 180–184.

Issa, J.P. (2004) CpG island methylator phenotype in cancer. *Nat Rev Cancer* 4: 988–993.

Issa, J.P. (2007) DNA methylation as a therapeutic target in cancer. *Clin Cancer Res* 13: 1634–1637.

Issa, J.P., Garcia-Manero, G., Giles, F.J., Mannari, R., Thomas, D., Faderl, S. *et al.* (2004) Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood* 103: 1635–1640.

Issa, J.P., Gharibyan, V., Cortes, J., Jelinek, J., Morris, G., Verstovsek, S. *et al.* (2005) Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J Clin Oncol* 23: 3948–3956.

Jones, P.A. and Baylin, S.B. (2007) The epigenomics of cancer. *Cell* 128: 683–692.

Karran, P. and Hampson, R. (1996) Genomic instability and tolerance to alkylating agents. *Cancer Surveys* 28: 69–85.

Kartalou, M. and Essigmann, J.M. (2001) Recognition of cisplatin adducts by cellular proteins. *Mutat Res* 478: 1–21.

Kato, K., Iida, S., Uetake, H., Takagi, Y., Yamashita, T., Inokuchi, M. *et al.* (2008) Methylated TMS1 and DAPK genes predict prognosis and response to chemotherapy in gastric cancer. *Int J Cancer* 122: 603–608.

Konstantinopoulos, P.A., Spentzos, D. and Cannistra, S.A. (2008) Gene-expression profiling in epithelial ovarian cancer. *Nat Clin Pract Oncol* 5: 577–587.

Lachner, M., O'Sullivan, R.J. and Jenuwein, T. (2003) An epigenetic road map for histone lysine methylation. *J Cell Sci* 116: 2117–2124.

Lehmann, U., Celikkaya, G., Hasemeier, B., Langer, F. and Kreipe, H. (2002) Promoter hypermethylation of the death-associated protein kinase gene in breast cancer is associated with the invasive lobular subtype. *Cancer Res* 62: 6634–6638.

Li, M., Balch, C., Montgomery, J.S., Jeong, M., Chung, J.H., Yan, P. *et al.* (2009) Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. *BMC Med Genomics* 2: 34.

Luqmani, Y.A. (2005) Mechanisms of drug resistance in cancer chemotherapy. *Med Princ Pract* 14(Suppl 1): 35–48.

Marks, P.A. and Xu, W.S. (2009) Histone deacetylase inhibitors: Potential in cancer therapy. *J Cell Biochem* 107: 600–608.

Matei, D.E. and Nephew, K.P. (2010) Epigenetic therapies for chemoresensitization of epithelial ovarian cancer. *Gynecol Oncol* 116: 195–201.

Miranda, T.B., Cortez, C.C., Yoo, C.B., Liang, G., Abe, M., Kelly, T.K. *et al.* (2009) DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol Cancer Ther* 8: 1579–1588.

Moreland, N.J., Illand, M., Kim, Y.T., Paul, J. and Brown, R. (1999) Modulation of drug resistance mediated by loss of mismatch repair by the DNA polymerase inhibitor aphidicolin. *Cancer Res* 59: 2102–2106.

Mund, C., Brueckner, B. and Lyko, F. (2006) Reactivation of epigenetically silenced genes by DNA methyltransferase inhibitors: basic concepts and clinical applications. *Epigenetics* 1: 7–13.

Olsen, E.A., Kim, Y.H., Kuzel, T.M., Pacheco, T.R., Foss, F.M., Parker, S. *et al.* (2007) Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 25: 3109–3115.

Papouli, E., Cejka, P. and Jiricny, J. (2004) Dependence of the cytotoxicity of DNA-damaging agents on the mismatch repair status of human cells. *Cancer Res* 64: 3391–3394.

Plumb, J.A., Steele, N., Finn, P.W. and Brown, R. (2004) Epigenetic approaches to cancer therapy. *Biochem Soc Trans* 32: 1095–1097.

Plumb, J.A., Strathdee, G., Sludden, J., Kaye, S.B. and Brown, R. (2000) Reversal of drug resistance in human tumor xenografts by 2′-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. *Cancer Res* 60: 6039–6044.

Press, J.Z., De Luca, A., Boyd, N., Young, S., Troussard, A., Ridge, Y. *et al.* (2008) Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. *BMC Cancer* 8: 17.

Ramalingam, S.S., Maitland, M.L., Frankel, P., Argiris, A.E., Koczywas, M., Gitlitz, B. *et al.* (2010) Carboplatin and Paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. *J Clin Oncol* 28: 56–62.

Shimodaira, H., Yoshioka-Yamashita, A., Kolodner, R.D. and Wang, J.Y. (2003) Interaction of mismatch repair protein PMS2 and the p53-related transcription factor p73 in apoptosis response to cisplatin. *Proc Natl Acad Sci U S A* 100: 2420–2425.

Steele, N., Finn, P., Brown, R. and Plumb, J.A. (2009) Combined inhibition of DNA methylation and histone acetylation enhances gene re-expression and drug sensitivity in vivo. *Br J Cancer* 100: 758–763.

Stewart, D.J., Issa, J.P., Kurzrock, R., Nunez, M.I., Jelinek, J., Hong, D. *et al.* (2009) Decitabine effect on

tumor global DNA methylation and other parameters in a phase I trial in refractory solid tumors and lymphomas. *Clin Cancer Res* 15: 3881–3888.

Stojic, L., Mojas, N., Cejka, P., Di Pietro, M., Ferrari, S., Marra, G. *et al.* (2004) Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev* 18: 1331–1344.

Stresemann, C., Brueckner, B., Musch, T., Stopper, H. and Lyko, F. (2006) Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines. *Cancer Res* 66: 2794–2800.

Suva, M.L., Riggi, N., Janiszewska, M., Radovanovic, I., Provero, P., Stehle, J.C. *et al.* (2009) EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer Res* 69: 9211–9218.

Tan, J., Yang, X., Zhuang, L., Jiang, X., Chen, W., Lee, P.L. *et al.* (2007) Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev* 21: 1050–1063.

Tang, X., Khuri, F.R., Lee, J.J., Kemp, B.L., Liu, D., Hong, W.K. *et al.* (2000) Hypermethylation of the death-associated protein (DAP) kinase promoter and aggressiveness in stage I non-small-cell lung cancer. *J Natl Cancer Inst* 92: 1511–1516.

Tang, X., Wu, W., Sun, S.Y., Wistuba, I.I., Hong, W.K. and Mao, L. (2004) Hypermethylation of the death-associated protein kinase promoter attenuates the sensitivity to TRAIL-induced apoptosis in human non-small cell lung cancer cells. *Mol Cancer Res* 2: 685–691.

Taniguchi, T., Tischkowitz, M., Ameziane, N., Hodgson, S.V., Mathew, C.G., Joenje, H. *et al.* (2003) Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. *Nat Med* 9: 568–574.

Teodoridis, J.M., Hall, J., Marsh, S., Kannall, H.D., Smyth, C., Curto, J. *et al.* (2005) CpG island methylation of DNA damage response genes in advanced ovarian cancer. *Cancer Res* 65: 8961–8967.

Varambally, S., Dhanasekaran, S.M., Zhou, M., Barrette, T.R., Kumar-Sinha, C., Sanda, M.G. *et al.* (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419: 624–629.

Wang, D. and Lippard, S.J. (2005) Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 4: 307–320.

Warrener, R., Beamish, H., Burgess, A., Waterhouse, N.J., Giles, N., Fairlie, D. *et al.* (2003) Tumor cell-selective cytotoxicity by targeting cell cycle checkpoints. *FASEB J* 17: 1550–1552.

Wood, L.D., Parsons, D.W., Jones, S., Lin, J., Sjoblom, T., Leary, R.J. *et al.* (2007) The genomic landscapes of human breast and colorectal cancers. *Science* 318: 1108–1113.

Xu, W.S., Parmigiani, R.B. and Marks, P.A. (2007) Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 26: 5541–5552.

Yamaguchi, S., Asao, T., Nakamura, J., Ide, M. and Kuwano, H. (2003) High frequency of DAP-kinase gene promoter methylation in colorectal cancer specimens and its identification in serum. *Cancer Lett* 194: 99–105.

Yang, N., Kaur, S., Volinia, S., Greshock, J., Lassus, H., Hasegawa, K. *et al.* (2008) MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. *Cancer Res* 68: 10307–10314.

Yoo, C.B. and Jones, P.A. (2006) Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov* 5: 37–50.

Yoshioka, K., Yoshioka, Y. and Hsieh, P. (2006) ATR kinase activation mediated by MutSalpha and MutLalpha in response to cytotoxic O6-methylguanine adducts. *Mol Cell* 22: 501–510.

Yu, J., Rhodes, D.R., Tomlins, S.A., Cao, X., Chen, G., Mehra, R. *et al.* (2007) A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. *Cancer Res* 67: 10657–10663.

Zhang, S., Balch, C., Chan, M.W., Lai, H.C., Matei, D., Schilder, J.M. *et al.* (2008) Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res* 68: 4311–4320.