Epigenetic approaches to cancer therapy

J.A. Plumb*, N. Steele*, P.W. Finn† and R. Brown*
†CRUK Centre for Oncology and Applied Pharmacology, Beatson Laboratories, University of Glasgow, Garscube Estate, Glasgow G61 1BD, U.K., and †TopoTarget Prolifix, Abingdon, OX14 4RY, U.K.

Abstract
Histone deacetylation and DNA methylation have a central role in the control of gene expression, including transcriptional repression of tumour suppressor genes. Loss of DNA mismatch repair due to methylation of the hMLH1 gene promoter results in resistance to cisplatin in vitro and in vivo. The cisplatin-resistant cell line A2780/cp70 is 8-fold more resistant to cisplatin than the non-resistant cell line, and has the hMLH1 gene methylated. Treatment with an inhibitor of DNA methyltransferase, DAC (2-deoxy-5′-azacytidine), results in a partial reversal of DNA methylation, re-expression of MLH1 (mutL homologue 1) and sensitization to cisplatin both in vitro and in vivo. PXD101 is a novel hydroxamate type histone deacetylase inhibitor that shows antitumour activity in vivo and is currently in phase I clinical evaluation. Treatment of A2780/cp70 tumour-bearing mice with DAC followed by PXD101 results in a marked increase in the number of cells that re-express MLH1. Since the clinical use of DAC may be limited by toxicity and eventual re-methylation of genes, we suggest that the combination of DAC and PXD101 could have a role in increasing the efficacy of chemotherapy in patients with tumours that lack MLH1 expression due to hMLH1 gene promoter methylation.

Introduction
Epigenetic inactivation of genes crucial for control of normal cell growth is a hallmark of cancer cells [1]. These epigenetic mechanisms include crosstalk between DNA methylation, histone modification and other components of chromatin higher-order structure leading to regulation of gene transcription. The transfer of a methyl group to the C-5 position of cytosines, almost always in the context of CpG dinucleotides, is the only known epigenetic modification of DNA itself. Many tumours show increased methylation of CpG islands, CpG-rich regions of DNA usually associated with gene promoters, which is associated with epigenetic gene silencing [2]. Genes aberrantly methylated in human tumours include tumour suppressor genes, genes involved in control of the cell cycle, apoptosis and drug sensitivity.

The MLH1 (mutL homologue 1) protein, part of the human DNA MMR (mismatch repair) system, has been shown to be important in determining sensitivity to a number of important chemotherapeutic agents [3,4]. Loss of MMR due to methylation of the bMLH1 gene promoter results in resistance to cisplatin in cell lines in vitro and in human tumour xenografts in vivo [5]. Methylation of the bMLH1 gene promoter is observed in many tumour types and is associated with clinical drug resistance in breast and ovarian cancers [6–9].

We have shown that treatment with the DNA-hypo-methylating agent DAC (2′-deoxy-5′-azacytidine) results in partial reversal of DNA methylation, re-expression of MLH1 and sensitization to cisplatin both in vitro and in vivo [5]. However, the clinical use of DAC may be limited by myelosuppression and the eventual re-methylation of genes. Baylin and coworkers [10] have shown that the combination of DAC and the histone deacetylase inhibitor TSA (trichostatin A) is more effective at reactivating transcription of epigenetically silenced genes such as MLH1 in tumour cell lines than either drug alone. We have investigated whether this drug combination results in increased expression of MLH1 and drug sensitivity of cells in vitro.

Methods
Cell lines LS274 and LS310 are small-cell lung cancer cell lines established from the same patient before treatment (LS274) and at relapse following chemotherapy (LS310). The methylation status of the bMLH1 gene promoter was determined by methylation-specific PCR essentially as described in [11]. A2780/cp70 is an in vitro derived cisplatin-resistant ovarian cancer cell line. The bMLH1 gene promoter is methylated in A2780/cp70 and it is therefore MMR-deficient and does not express MLH1 [5]. Media, growth conditions and drug sensitivity testing were as described in [5,12]. For the animal studies, A2780/cp70 cells were injected subcutaneously into the right flank of athymic nude mice (CD1 nu/nu). Once tumours were visible, groups of three mice were injected intra-peritoneally with either PBS or DAC (5 mg/kg at 3 h intervals for a total of three injections). After 72 h, mice were injected intra-peritoneally with either DMSO (10% in water) or PXD101 (40 mg/kg). After a further 6 days, tumours were removed, fixed and stained for MLH1 expression as described previously [5]. Animal studies were carried out under an appropriate UK Home Office Project...
Results and discussion

The hMLH1 gene promoter is methylated in LS310 (post-treatment) consistent with the reduced expression of MLH1 (2-fold) and with the relative resistance to cisplatin of LS310 (1.8-fold; \( P < 0.001 \)) when compared with LS274 (pre-treatment; Figure 1). Treatment of LS310 with DAC (0.2 \( \mu M \)) resulted in a 1.4-fold increase in MLH1 expression and a 1.5-fold increase in sensitivity to cisplatin (\( P < 0.01 \); Figure 1C). Treatment with DAC (0.2 \( \mu M \)) and TSA (300 nM) together resulted in an increase in both MLH1 expression (1.9-fold) and cisplatin sensitivity (2-fold; \( P < 0.01 \)) to levels comparable with that of LS274. TSA alone had no effect on MLH1 expression. As expected, none of the treatments had any significant effect on expression of MLH1 and drug sensitivity of LS274.

These observations are consistent with the observations of Baylin and co-workers [10] and show clearly that the combination of DNA demethylation and histone deacetylase inhibition acts synergistically to re-express silenced genes and, in the case of MLH1, this results in enhanced drug sensitivity \textit{in vitro}. Since TSA is not suitable for use in patients, we have also investigated whether the novel hydroxamate type histone deacetylase inhibitor PXD101 [13], currently in phase I clinical evaluation, can synergize with DAC \textit{in vivo} to induce re-expression of MLH1. Interestingly, we were able to reproduce the effects on gene expression \textit{in vivo} in a cisplatin-resistant human tumour xenograft model (Figure 2). The drug schedule and tumour model used for the mouse studies (DAC on day 1 and PXD101 on day 3) were based on our previous observation with DAC alone, where demethylation of the hMLH1 gene promoter was maximal by 9 days but was clearly apparent as early as 3 days after DAC treatment [5]. A2780/cp70 tumours from mice treated with PXD101 3 days after DAC treatment showed a marked increase in MLH1 expression when compared with those treated with DAC alone (Figure 2C, cf. Figure 2D). PXD101 alone had no effect on gene expression (Figure 2B) and we have already shown that this dose of PXD101 can be administered to mice daily for up to 7 days with no apparent toxicity [13].

A clinical phase I trial is in progress to determine whether it is possible to use DAC in the clinic to increase the efficacy of chemotherapy in patients whose tumours lack MLH1 expression due to gene promoter methylation [14].
the phase I trial is at an early stage, we have already shown a reduction in 5-methyl-2′-deoxycytosine levels in blood cells of patients, which is at least equivalent to that observed in mice [15]. Our results now suggest that, with the correct scheduling, the combination of DAC and PXD101 is a rational approach to improve the efficacy of chemotherapy in patients with tumours that lack MLH1 expression due to hMLH1 gene promoter methylation.

References

Received 5 July 2004