CpG-island methylation and epigenetic control of resistance to chemotherapy

J.M. Teodoridis, G. Strathdee, J.A. Plumb and R. Brown
Centre for Oncology and Applied Pharmacology, Cancer Research UK Beatson Laboratories, University of Glasgow, Glasgow, U.K.

Abstract
Aberrant methylation of CpG islands (CpG-rich regions of DNA associated with the promoters of many genes) is associated with transcriptional inactivation of genes involved in tumour development. Genes involved in key DNA damage response pathways, such as cell-cycle control, apoptosis signalling and DNA repair can frequently become epigenetically silenced and methylated in tumours. This may lead to differences in intrinsic sensitivity of tumours to chemotherapy, depending on the specific function of the gene inactivated. Furthermore, chemotherapy itself may exert a selective pressure on epigenetically silenced drug sensitivity genes present in subpopulations of cells, leading to acquired chemoresistance. Clinical trials of epigenetic therapies are now in progress, and epigenetic profiling using DNA methylation will provide guidance on optimization of the use of these therapies with conventional chemotherapy, as well as helping to identify patient populations who may particularly benefit from such approaches.

DNA methylation, the addition of a methyl group to the carbon-5 position of cytosine residues, is the only common covalent modification of human DNA and occurs almost exclusively at cytosine residues that are followed immediately by a guanine (so-called CpG dinucleotides) [1]. In the bulk of the genome, CpG dinucleotides are relatively rare and are nearly always methylated. In contrast, small stretches of DNA, known as CpG islands, are rich in CpG nucleotides and in normal cells are nearly always methylation-free. These CpG islands are frequently associated with the promoter regions of human genes and methylation within the islands has been shown to be associated with post-translational modification of histones, chromatin condensation and transcriptional inactivation of the associated gene.

Aberrant methylation of CpG islands is frequently observed in tumours compared with normal tissue and for many tumours is an early event during tumorigenesis. It is estimated that in tumours there are, on average, 600 CpG islands aberrantly methylated, although this can vary widely between tumour types and within particular histological subtypes [2]. Moreover, methylation does not occur randomly, as certain CpG islands are consistently methylated in several tumour types, whereas other CpG islands are predominantly methylated only in specific tumour types [3]. This is consistent with a model in which methylation of CpG islands at particular genes would give the cancer cell a growth or survival advantage and so patterns of methylation emerge depending on the selective pressure for gene silencing in the tumour type examined. A large number of genes have now been identified that are targeted by aberrant methylation in tumours, including genes involved in essentially all facets of tumour development and progression (Table 1). Indeed, for many important genes, such as MLH1, BRCA1 and E-cadherin, aberrant methylation is by far the most frequent mechanism associated with inactivation of these genes in sporadic tumours.

In addition to methylation and silencing of specific genes involved in tumorigenesis, it has been suggested that tumours may acquire a methylator phenotype [4]. It is possible that some genes are becoming methylated by chance and co-selected during tumour development, despite having no immediate effect on tumour phenotype. However, such changes may influence subsequent behaviour of the tumour by influencing biological properties, such as propensity to undergo invasion and metastasis or sensitivity to treatment with chemotherapy. Consistent with this, we have shown previously that late-stage ovarian cancers can be clustered into two groups based on differences in CpG island methylation [5]. Increased methylation of a subset of CpG islands in these tumours significantly correlated with worse clinical outcome as defined by the time of clinical disease recurrence after chemotherapy [5].

These types of studies raise the possibility of using methylation profiling to identify which patients may benefit more from existing treatments, or identifying patient populations probably suitable for clinical trials of novel agents, which target epigenetic mechanisms. We are, therefore, now examining whether genes involved in response of cells to DNA damage, including genes involved in DNA repair, cell-cycle control and apoptosis, can become aberrantly methylated in stage III and IV epithelial ovarian tumours surgically removed before chemotherapy and whether this can predict clinical outcome.

Many reports have been published on potential drug resistance markers in ovarian cancer, mainly derived from the study of acquired resistance in experimental models [6]. However, most clinical studies of drug resistance have focused...
Table 1 | Examples of genes frequently methylated and epigenetically silenced in tumours

<table>
<thead>
<tr>
<th>Cancer-associated pathway</th>
<th>Gene</th>
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<tbody>
<tr>
<td>Limitless replicative potential</td>
<td>Rb, CDX1, GATA-4, GATA-5, Myf-3, SOCS-3</td>
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<tr>
<td>Insensitivity to anti-growth signals</td>
<td>CyclinD2, ErA, INHA, L01, MB-Comt, p15INK4A, p16INK4a, p21WAF1, p27KIP1, p57KIP2, Pox5, Pten, RARβ, RASSF1, STAP5, TGIFR1</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>THBS1, THBS2, VHL</td>
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<tr>
<td>Evasion of apoptosis</td>
<td>Apaf1, Cosp8, DAPK, DCC-1, Fas, p14ARF, p53, P73, SHP1, Traf-1, XAF1</td>
</tr>
<tr>
<td>Intracellular adhesion and tissue invasion</td>
<td>ADAM23, E-Cadherin, H-Cadherin, Cov-1, CD44, CLCA2, CLDN-7, gelosin, laminin-5, OPCML, TIMP3, SLIT2, TFF1, TSLC1</td>
</tr>
<tr>
<td>DNA repair</td>
<td>MGMT, MLH1, BRCA1, FanCF</td>
</tr>
<tr>
<td>Carcinogen metabolism</td>
<td>GSTP1, CyP4501A1, MDR1, RFC</td>
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on tumour characteristics at presentation, rather than at relapse. Although studies of tumours prechemotherapy are important for identifying prognostic markers and possible mechanisms of intrinsic resistance, they will provide limited information on mechanisms of acquired resistance. Thus tumours at presentation will be heterogeneous, consisting of chemosensitive and resistant subpopulations, making it difficult to identify the subpopulations that lead to treatment failure of an initially responsive tumour. Since chemotherapy positively selects for resistant subpopulations, analysis of tumours at relapse may allow these subpopulations of cells to become more apparent and will allow mechanisms of acquired, rather than intrinsic, drug resistance to be identified and analysed for associations with patient survival.

Owing to the difficulties in obtaining tumour samples routinely, especially from patients at relapse, and for ease of sample collection in the context of large, multicentre clinical trials, there has been increasing interest in the use of markers in plasma and serum for the prognostication and monitoring of cancer [7]. DNA can be detected in plasma from cancer patients with the same characteristic changes, including CpG-island methylation, found in the corresponding tumour [8]. For ovarian cancer, such changes have been detected with high specificity and have been suggested as a diagnostic tool [9,10]. DNA methylation is particularly suited for such analysis of plasma DNA since sensitive methylation-specific PCR-based assays require only small amounts of DNA and methylation of genes frequently aberrantly methylated in tumours is rarely observed in normal tissue, including peripheral blood mononuclear cells DNA, which may be present with tumour DNA in plasma [11].

We have examined plasma DNA of patients with epithelial ovarian cancer enrolled in the SCOTROC1 Phase III clinical trial for methylation of the hMLH1 CpG-island before carboplatin/taxoid chemotherapy and at relapse [12]. Methylation of hMLH1 is increased at relapse, with 25% (34/138) of relapse samples having hMLH1 methylation, which is not detected in matched prechemotherapy plasma samples. Furthermore, hMLH1 methylation is significantly associated with increased microsatellite instability in plasma DNA at relapse, providing an independent measure of function of the MMR pathway. Acquisition of hMLH1 methylation in plasma DNA at relapse predicts poor overall survival of patients, independent from time to progression and age (hazard ratio, 1.99, 95% CI 1.20–3.30, P = 0.007). These results support the clinical relevance of acquired hMLH1 methylation, and concomitant loss of DNA mismatch repair, after chemotherapy of ovarian cancer patients.

The prevalence of aberrant methylation of genes in tumours makes it an attractive target for novel anti-cancer therapies. Several small molecules that are potential epigenetic therapies are now entering early clinical trials. We have a number of clinical trials of the demethylating agent 5′-deoxy-5-azacytidine (Decitabine) in combination with carboplatin and other cytotoxics currently in progress. Epigenetic analysis of patterns of global DNA methylation may help to identify those patients who will particularly benefit from these types of treatments.

References

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