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Demethylation of the *MCJ* gene in stage III/IV epithelial ovarian cancer and response to chemotherapy

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Abstract

Objective. Methylation of a CpG island within the *Methylation controlled DNAJ* (*MCJ*) gene results in loss of expression in normal and neoplastic cells. Normal ovarian surface epithelial cells are methylated at the *MCJ* CpG island and do not express the MCJ gene. Furthermore, re-expression of the *MCJ* gene, in ovarian cancer cell lines, has been correlated with increased sensitivity to several important chemotherapeutic drugs. The objective of this study was to determine the extent of *MCJ* promoter methylation in epithelial ovarian cancer patients and address the possible role of *MCJ* methylation levels in response to chemotherapy in ovarian cancer patients.

Methods. The methylation status of 35 CpG sites within the *MCJ* CpG island was determined by sequencing of sodium bisulfite modified tumor DNA in 41 patients with stage III/IV epithelial ovarian tumors. Levels of methylation of the MCJ CpG island were then compared with response to therapy and overall survival in the patients.

Results. The analysis identified frequent loss of *MCJ* methylation in ovarian tumors, with only a subset retaining high methylation levels. While 93% (38/41) of tumors examined showed some level of *MCJ* methylation, only 17% (7/41) retained very high levels (>90% methylation). The presence of such high levels of CpG island methylation correlated significantly with poor response of patients' tumors to therapy ($P = 0.027$) and poor overall survival ($P = 0.023$, hazard ratio = 2.9).

Conclusions. These results suggest that *MCJ* methylation may be useful as a marker of response to chemotherapy in ovarian cancer and are consistent with previous in vitro data linking loss of MCJ expression with drug resistance.

Keywords: DNA methylation; Drug resistance; Ovarian cancer; CpG island; MCJ

Introduction

In recent years, it has become clear that epigenetic changes, particularly alterations in DNA methylation and histone acetylation, play a key role in the genesis and progression of human cancer and such epigenetic changes are now important targets for the development of novel therapeutic approaches [1]. DNA methylation occurs almost exclusively at CpG dinucleotides (cytosine residues immediately followed by guanines) and about 70% of CpG sites in the human genome are methylated [2]. CpG dinucleotides are under-represented throughout genome, with the exception of short stretches of DNA known as CpG islands. These CpG islands are GC-rich stretches of DNA of up to a few kilobases in length with close to the expected number of CpG dinucleotides and are frequently associated with human genes, often mapping to the promoter/first exon of the gene. In contrast to the bulk of DNA, the CpG sites within CpG islands are almost always methylation free [2]. Essentially all tumor types exhibit gross alterations in the pattern of DNA methylation, with aberrant methylation of CpG islands being observed at up to several thousand different loci in a single

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tumor [3]. Hypermethylation of CpG islands located within the 5′ region of genes is known to lead to transcriptional repression [4] and genes known to be critical both in tumor development and also in the response of tumors to therapy are known to be inactivated by this mechanism [3].

The *methylation controlled DNA J* (*MCJ*) gene was originally identified by Shridhar and colleagues [5]. Transfection of the gene back into MCJ-deficient cell lines had no apparent effect on cell growth but rendered the cells more sensitive to a number of important chemotherapeutic agents, including cisplatin and paclitaxel, which are the mainstays of chemotherapeutic treatment for ovarian cancer patients [6]. Consistent with this observation, we have found loss of expression of MCJ in eight out of ten independently derived cisplatin-resistant derivatives of the ovarian carcinoma cell line A2780 [7].

We have recently identified a CpG island within the *MCJ* gene, which begins within the 1st exon of *MCJ* and extends into the first intron [7]. Even though the CpG island does not overlap the gene promoter, hypermethylation of the island results in transcriptional repression of the gene and reversal of this DNA methylation results in gene re-expression. Although there are also a limited number of CpG sites within the *MCJ* promoter, expression of MCJ is independent of the methylation status of these promoter CpG sites [7], indicating that methylation within the CpG island, and not the promoter region, is critical in silencing of the *MCJ* gene. In addition, methylation of the CpG island is associated with reduced levels of histone acetylation [7], consistent with current models of DNA methylation conferring transcriptional silencing through binding of MBD (methyl binding domain) protein containing complexes and subsequent chromatin remodeling [4]. These results suggest that the DNA methylation status of the *MCJ* CpG island could influence gene expression in ovarian tumor cells and lead to alterations in response to chemotherapy. To begin to address this question, we have examined the methylation status of the *MCJ* CpG island (at a total of 35 CpG sites) in a study of 41 retrospectively collected stage III/IV ovarian tumors by sequencing of bisulfite modified DNA. This analysis demonstrated widely variable levels of CpG island methylation in the ovarian tumors and identified a correlation between patients whose tumors maintained high levels of *MCJ* methylation, which would be predicted to result in very low levels of *MCJ* gene expression, with poor response to chemotherapy and reduced overall survival. These results are consistent with a role for *MCJ* in determining chemosensitivity in vivo.

Materials and methods

Tissue samples

Ovarian tumor samples were obtained from the Western Infirmary and Stobhill General hospitals, Glasgow, UK and Pembury Hospital, Kent, UK. Ethical approval for all samples collected had been obtained and samples were collected according to MRC operational and ethical guidelines on “Human tissue and biological samples for use in research”. The tumor specimens underwent gross examination, sampling and microscopy by an experienced histopathologist. The tissue submitted for research purposes was grossly composed of tumor only. All samples were stored frozen at −70°C. Pathology reports, including histological subtype and grade, were obtained where possible. Genomic DNA was extracted for methylation analysis as previously described [8]. For estimation of tumor cell content, tissue samples were embedded in paraffin blocks and 5-μM sections were stained with hematoxylin and eosin. The percentage of tumor cells present in each section was assessed by a histopathologist, blind to the methylation status of the samples. Response to therapy, defined by lesion size using the RECIST criteria, was obtained from the patient’s case notes in a retrospective manner, and was done in an anonymized fashion by data managers from the Beatson Oncology Centre and Stobhill Hospital, Glasgow. All patients were treated with either cisplatin or carboplatin and the majority were also treated with a taxoid.

Bisulfite sequencing

DNA was modified with sodium bisulfite using the CpGenome modification kit (Chemicon; Hampshire, UK) as per the manufacturer’s instructions. All samples were resuspended in 40 μl of TE and 1 μl of this was used for subsequent PCR reactions. The samples were amplified in 25-μl volumes containing 1× manufacturer’s buffer, 1 unit of FastStart taq polymerase (Roche; Lewes, UK), 4 mM $MgCl_2$, 10 mM dNTPs, and 75 ng of each primer. PCR was performed with one cycle of 95°C for 6 min, 35 cycles of 95°C for 30 s, 63°C for 30 s, and 72°C for 30 s, followed by one cycle of 72°C for 5 min. The primers used for the PCR reactions (and positions relative to the transcriptional start site) for the CpG island were: *MCJ* CpG—forward (+122 bp) 5′-GTTTGGGGAGGGATTAGG-3′, reverse (+547 bp) 5′-CTAACAAAACTCACCAATCTCTAC-3′. All PCR reactions were carried out on a PTC-225 DNA engine tetrad (MJ Research; Watertown, Ma). PCR products were isolated using GenElute agarose spin columns (Sigma, Poole, UK), as per manufacturer’s protocol and cloned using the PGem-T vector system II PCR cloning vector (Promega; Southampton, UK). White (insert containing) colonies were picked and grown up in 4 ml of LB containing 100 μg/ml ampicillin and plasmid DNA isolated using a QIAprep Spin miniprep kit (Qiagen; Crawley, UK). Sequencing was then carried out on a CEQ 2000XL DNA analysis system (Beckman Coulter; High Wycombe, UK) using the Sp6 or T7 sequencing primers. The accuracy of the quantitation of methylation