5-MeC PRECISE QUANTITATION IN GENOMIC DNA AND SPECIFIC SEQUENCES: RELEVANCE IN CISPLATIN RESISTANCE

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Cisplatin is a widely used chemotherapeutic drug, whose major drawback is patient resistance due to, among others, changes in genome methylation profiles. Cytosine methylation at CpG sites is one of the most important epigenetic marks in vertebrates, and seems to be related to cisplatin resistance when studied at promoter regions of specific genes. However, the relationship between global DNA methylation and cisplatin resistance is not clear.

We have developed a novel technique to precisely quantitate 5-methyl-2′-deoxycytidine (5-MeC), based on high performance liquid chromatography (HPLC) followed by ultraviolet absorbance detection, and used it to study methylation both in genomic DNA and in specific sequences, which were isolated with streptavidin coated magnetic beads and 5'-biotinated oligonucleotides. Cisplatin sensitive (A2780) and resistant (A2780cis) human cell lines, treated for 3h, with 40 μM, were used for this analysis. Fragments of 700-1000 bp size, from the promoter regions of *BAX*, *CASP3*, *BCL2*, *TP53*, *ERCC1*, *ERCC4*, and *POLQ* genes, were selected and their methylation levels were analyzed both in treated and untreated cells. Bisulfite pyrosequencing was used for comparisons. Gene expression was determined, with quantitative RT-PCR using Taqman probes, to evaluate possible relationships with promoter methylation.

Results revealed that: (i) cisplatin treatment increased global genome methylation in both cell lines; (ii) methylation levels between 5 and 29% were detected for all the analyzed promoters, except for that of *ERCC4* gene; (iii) methylation differences between cell lines were detected for *BAX* and *ERCC1* promoters; and (iv) treatment effects on methylation profile were found for *BAX* in A2780cis cells. Moreover, the new method seems to be

more sensitive than bisulfite pyrosequencing. Finally, the detected methylation profiles of *BAX* promoter were apparently related to changes in gene expression.