tumor suppressor genes (TSG) in many types of cancers, including lung cancer. However, their expression can be restored by demethylating and histone deacetylating inhibiting drugs such 5Aza-dC-2deoxycytidine (5Aza-dC) and trichostatin A (TSA). Platinum-induced DNA hypermethylation may be involved in the development of drug-resistant phenotypes by inactivating genes required for drug cytotoxicity. We aim to identify the global profile of TSG silenced by epigenetic mechanisms in NSCLC cell lines after CDDP treatment, and therefore potentially involved in the development of chemotherapy resistance. The present study is based on an expression microarray analysis of genes reactivated in a set of CDDP-resistant and sensitive NSCLC cell lines after 5Aza-dC and TSA treatment. CDDP-resistant cells were established by treating two NSCLC cell lines, H-460 and H-23, with increasing concentrations of CDDP. Then, cells were exposed to 5Aza-dC (5μM) and TSA (500nM) before RNA extraction. Total RNA from the different cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome. We selected for validation those genes upregulated after 5Aza-dC and TSA treatment, which expression was previously downregulated in CDDP-resistant versus cisplatin-sensitive cell lines. Next, we confirmed the presence of CpG island in the promoter region, the expression in normal lung cells and excluded those genes located in imprinted areas. Gene expression changes were confirmed by semi-quantitative RT-PCR. Promoter methylation was validated by bisulfite sequencing. Finally, methylation of validated genes was analyzed by methylation specific PCR (MSP) in NSCLC specimens with known CDDP response. Epigenetic regulation of selected genes was further studied in the abstract presented by M Cortes (back to back Poster). We have identified, a panel of genes with altered expression as a result of CDDP and epigenetic reactivation treatments. After validation of five, we confirmed one gene as a potential clinical marker, able to detect with 80% specificity, sensitive versus CDDP-resistant tumors in a panel of 30 paraffin embedded NSCLC samples. This study provides information regarding de novo promoter hypermethylation of potential TSG involved in the development of resistance and their potential use as targets enabling the diagnosis and chemotherapy treatment of NSCLC to be approached at the molecular level.Supported by Health Investigation funding (FIS/ISCIII). Supprted by FIS project number: PI061234 and by an unrestricted educational grant by Fundación Mutua Madrileña

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Differential expression of DNAmethyltransferases in sensitive versus cisplatin resistant NSCLC cell lines

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Aberrant promoter hypermethylation is a common epigenetic mechanism for the silencing of tumor suppressor genes (TSG) in many types of cancers, including lung cancer. In eukaryotes there are three families of DNA methyltransferase enzymes (DNMT) that catalyzed the DNA methylation process. DNMT3 family is involved primarily in methylation of new sites and the DNMT3B member is more highly expressed in human cancer cell lines and primary tumors than in normal tissue. In addition it has been recently reported a new subfamily of DNMT3B (delta-DNMT3B) that are the predominant forms in non-small cell lung cancer (NSCLC), suggesting an important role in DNA methylation control in lung tumorogenesis. One of the main problems in this tumor type is the frequent development of acquired-chemotherapy resistance. Genetic and epigenetic alterations are known to underlie the initiation and progression of neoplasia, therefore, one of the possible reasons for the development of chemotherapy resistance in NSCLC might be the epigenetic inactivation of certain TSG as a consequence of chemotherapy treatment. In addition, cisplatin (CDDP), the paradigm of cytotoxic drugs for NSCLC treatment, has been reported to induce, de novo DNA hypermethylation in vivo.

We analyzed the potential role of the DNMT family in the development of chemotherapy resistance to CDDP in NSCLC. The study is based on an expression microarray analysis of genes reactivated in a set of CDDP-resistant and sensitive NSCLC cell lines after 5Aza-dC and TSA treatment. Resistant cells were established by treating two NSCLC cell lines, H-460 and H-23, with increasing concentrations of CDDP. Then, cells were exposed to 5Aza-dC (5μM) and TSA (500nM) before RNA extraction in order to reactivate those genes epigenetically silenced in the resistant cell lines. Total RNA from the different cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome. The first part of this study is accessible in the abstract presented by I Ibanez de Caceres, in which we show very promising results regarding de novo promoter hypermethylation of specific genes and their relevance in the development of chemo-resistance to CDDP in NSCLC. In order to confirm a possible role of DNMT members silencing the selected genes in chemoresistance, we analyzed the differential expression of the DNMT family on sensitive versus CDDP-resistant cell lines. We found a marked increased expression of DNMT3B in the resistant cell lines compared with the parental ones. We confirmed this result by RT-PCR in both NSCLC cell lines, and in the ovarian human cancer cell lines 41M and 41MR, sensitive and resistant to CDDP respectively. Those results indicate a possible role of DNMT3B on the epigenetic regulation of specific genes responsible of the CDDP-acquire-resistance process; defining possible innovative treatment strategies for platinum resistant tumors.

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EGFR mutation in renal cell carcinoma confers sensitivity to gefitinib treatment

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Intragenic microdeletions and selected missense mutations located within tyrosine domain of epidermal growth factor receptor (EGFR) are known to be associated with the pronounced response to low molecular weight EGFR tyrosine kinase inhibitors (TKI), gefitinib or erlotinib. Unfortunately, these TKI-sensitizing mutations have been detected almost exclusively in lung adenocarcinomas, while their occurrence in tumors of other histological types or other organs is exceptionally rare. We applied EGFR mutation test as the last hope option to a heavily pretreated patient G., 60 years old, who suffered from the progression of renal cell carcinoma (RCC) and was administered to the hospital due to life-threatening condition. Unexpectedly, PCR and sequencing analysis revealed “lung-type” 15 base pair deletion, so the therapy by gefitinib was applied. This treatment led to a dramatic symptomatic response within the first week of therapy; the reduction of dyspnea was so evident that allowed the patient to return to the work. Clinical examination demonstrated complete disappearance of extensive pleuritis and pericarditis. Computed tomography measurement of metastatic lesions revealed minor response which could be classified as disease stabilization (RECIST criteria). The duration of clinical benefit was 4 months. The above observation led to a question, whether EGFR mutations occur at a noticeable frequency in RCC and whether their testing has to be considered in the routine clinical setting. Available literature indicates that only 38 RCC samples have been tested for the presence of EGFR mutations up to now, and one of those contained “lung-type” EGFR deletion. Therefore, we collected 118 RCC cases and subjected the tumor material to molecular analysis. However, none of these tumors contained TKI-sensitizing EGFR mutation. Taken together with published data, the following conclusions can be drawn from this study: 1) By now, 3 cases of gefitinib treatment of EGFR mutation-containing non-lung tumors (1 thymoma, 1 ovarian carcinoma, and 1 RCC from this study) have been reported, and all of them demonstrated evident clinical benefit from the therapy. Therefore, intragenic deletions of EGFR confer sensitivity to TKI treatment independently of the tumor type. 2) EGFR mutations in RCC are rare, thus the utility of the appropriate test for clinical management of kidney cancer remains questionable.

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MLH1 promotor hypermethylation, BRAF and K-ras mutation analysis on tumours suspected from Lynch Syndrome to prioritize mismatch repair gene testing

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Background: Microsatellite Instability (MSI) testing and immuno-histochemistry (IHC) are powerful tools that help identify individuals at risk for having LS and current diagnostic strategies can detect almost all highly penetrant Mismatch Repair (MMR) gene mutations. Our goals were to compare the performance of two panels of microsatellite markers in relation to IHC, as well as BRAF V600E mutation- and MLH1 promotor hypermethylation assays, and the determination of KRAS mutations in the Microsatellite Stable tumours (MSS).

Methods: Patients with a family history suggestive of Lynch syndrome (n=524) in the time period of 2005 till 2007 were tested for MSI and IHC staining of the MMR proteins MLH1, MSH2, MSH6 and PMS2. MSI-high tumours without expression of MLH1 in IHC, were tested for BRAF V600E