Genetic Variation Predicting Cisplatin Cytotoxicity Associated with Overall Survival in Lung Cancer Patients Receiving Platinum-Based Chemotherapy

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Abstract

**Purpose:** Inherited variability in the prognosis of lung cancer patients treated with platinum-based chemotherapy has been widely investigated. However, the overall contribution of genetic variation to platinum response is not well established. To identify novel candidate single nucleotide polymorphisms (SNP)/genes, we carried out a genome-wide association study (GWAS) for cisplatin cytotoxicity by using lymphoblastoid cell lines (LCL), followed by an association study of selected SNPs from the GWAS with overall survival (OS) in lung cancer patients.

**Experimental Design:** A GWAS for cisplatin was conducted with 283 ethnically diverse LCLs. A total of 168 top SNPs were genotyped in 222 small cell lung cancer (SCLC) and 961 non-SCLC (NSCLC) patients treated with platinum-based therapy. Association of the SNPs with OS was determined by using the Cox regression model. Selected candidate genes were functionally validated by siRNA knockdown in human lung cancer cells.

**Results:** Among 157 successfully genotyped SNPs, 9 and 10 SNPs were top SNPs associated with OS for patients with NSCLC and SCLC, respectively, although they were not significant after adjusting for multiple testing. Fifteen genes, including 7 located within 200 kb up or downstream of the 4 top SNPs and 8 genes for which expression was correlated with 3 SNPs in LCLs were selected for siRNA screening. Knockdown of DAPK3 and METTL6, for which expression levels were correlated with the rs11169748 and rs2440915 SNPs, significantly decreased cisplatin sensitivity in lung cancer cells.

**Conclusions:** This series of clinical and complementary laboratory-based functional studies identified several candidate genes/SNPs that might help predict treatment outcomes for platinum-based therapy of lung cancer. Clin Cancer Res; 17(17); 5801–11. ©2011 AACR.

Introduction

Platinum compounds are the major chemotherapeutic agents used to treat both small cell lung cancer (SCLC) and non-SCLC (NSCLC), with platinum compounds as the base for combination chemotherapy. Meta-analyses from randomized trials of cisplatin-based chemotherapy versus best supportive care have shown that cisplatin-based chemotherapy was associated with a modest improvement in overall survival (OS; ref. 1). Unfortunately, although these agents have shown success in treating lung cancer, the use of platinum-based chemotherapy is limited by the development of chemoresistance and toxicity. In addition, response rates vary greatly among individuals for any given cisplatin-based chemotherapy regimen. Factors such as disease stage, tumor histology, sex, tobacco exposure, and patient age may influence platinum-based chemotherapy outcomes. Although tumor DNA is important for drug response, previous studies have shown clearly that germ-line genetic variations also play an important role in determining treatment outcome (2–4). Therefore, identification and characterization of the role of genetic variation in differential response to platinum may help optimize individual treatment plans and improve platinum treatment outcomes.

Chemoresistance and toxicity associated with platinum-based therapy are complex phenotypes that may involve many processes (5). These processes include alterations in...
Translational Relevance

Chemotherapy with platinum-based regimens is the standard of care for both small cell and non–small cell lung cancer, but large interindividual variations are observed in platinum efficacy and toxicity. However, reliable genetic biomarkers for the prediction of response to platinum-based therapy are still not well established. Our study identified several genetic variants that are potentially associated with the efficacy of platinum-based chemotherapy in patients with lung cancer by using genome-wide analysis of data generated with lymphoblastoid cell lines, followed by genotyping studies using DNA samples from lung cancer patients and functional validation in lung cancer cells. These results provide important insight into mechanisms that might contribute to variation in response to platinum-based therapy of lung cancer.

drug influx or efflux, detoxification through glutathione conjugation, DNA repair capacity, and other cellular pathways required for proper response to DNA damage (6). The development of chemoresistance to platinum agents can be due to germline genetic variation or acquired mutation through their effects on mRNA and protein levels in key pharmacokinetic or pharmacodynamic pathways. Candidate gene and/or candidate pathway approaches indicated that single nucleotide polymorphisms (SNP) in genes responsible for drug influx and efflux (7), metabolism and detoxification (8–10), and DNA damage repair pathways (2–4, 11–17) were associated with treatment outcome of lung cancer patients. However, the overall contribution of these genetic biomarkers in the prediction of response to platinum-based therapy is still not well established, suggesting that additional genetic variants might be involved.

Therefore, in this study, we took a genome-wide approach by using 283 lymphoblastoid cell lines (LCL) to identify SNPs/genes that might contribute to variation in cisplatin cytotoxicity as represented by IC_{50} values. This step was followed by genotyping top candidate SNPs from the LCL genome-wide association study (GWAS) by using 1,183 DNA samples from lung cancer patients treated with platinum-based therapy to identify SNPs that might be associated with OS. Finally, functional studies of candidate genes identified during the association studies were carried out by using siRNA knockdown in human lung cancer cells.

Materials and Methods

Cell lines

This study was reviewed and approved by the Mayo Clinic Institutional Review Board. Human Variation Panel LCLs from sample sets HD100AA, HD100CAU, HD100CHI, corresponding to 100 African–American (AA), 100 Caucasian–American (CA), and 100 Han Chinese–American (HCA) unrelated subjects, were obtained from the Coriell Cell Repository. The National Institute of General Medical Sciences had obtained and anonymized these cell lines before deposit, and all subjects had provided written informed consent for the use of their samples for research purposes. Human NSCLC cell lines H1437, H1299, and the SCLC cell line H196 were obtained from the American Type Culture Collection. LCLs were cultured in RPMI 1640 medium (Mediatech) supplemented with 15% FBS (Mediatech), H1437, H1299, and H196 cell lines were cultured in RPMI 1640 medium containing 10% FBS.

Patient samples

The study group included 1,183 Caucasian patients with pathologically confirmed primary lung cancer, 222 SCLC and 961 NSCLC, who were treated with platinum-based chemotherapy at the Mayo Clinic between 1997 and 2008. Details with regard to clinical characteristics of patients, patient enrollment, diagnosis, and data collection procedures were described previously (18–20). Briefly, each patient was identified through the Mayo Clinic pathologic database. After written informed consent had been obtained, a blood sample was collected. The characteristics of patients, including demographics, lung cancer pathology, anatomic site, and types and timing of treatment and chemotherapeutic agents, were abstracted from patient medical records by a trained nurse. Vital status and cause of death were determined by reviewing the Mayo Clinic registration database and medical records, correspondence from patients’ next-of-kin, death certificates, obituary documents, the Mayo Clinic Tumor Registry, and the Social Security Death Index website. Clinical staging and recurrence or progression information was determined by results from available chest radiography, computerized tomography, bone scans, positron emission tomography scans, and magnetic response imaging. All patients were actively followed up during the initial 6 months after diagnosis, with subsequent annual follow-up by mailed questionnaires and annual verification of the patients’ vital status.

Cisplatin cytotoxicity assay

Cisplatin was obtained from Sigma-Aldrich and was dissolved in dimethyl sulfoxide (DMSO) immediately before use. Cells (4 × 10^4 cells per well) were plated into 96-well plates and incubated with cisplatin for 72 hours in 8 concentrations ranging from 0.1 to 80 μmol/L. DMSO alone was used as a control. Cisplatin cytotoxicity was evaluated by determining the concentration of cisplatin required to inhibit growth and/or survival by 50% (IC_{50}) by using the CellTiter 96@ Aqueous Non-Radioactive Cell Proliferation Assay (Promega). Experiments were conducted successfully for 283 LCLs (91 AA, 96 CA, and 96 HCA).

Genome-wide SNP and expression arrays for LCLs

The genotyping and expression array data for all LCLs were described previously (21–23) and are publicly available from NCBI Gene Expression Omnibus.
of 25 nmol/L of specific or negative control siRNAs and the mixture into 96-well plates, mixed with an siRNA mixture consisting of 6.0 Chip SNP data were also obtained for the same cell lines. SNPs that deviated from Hardy–Weinberg Equilibrium (HWE; ref. 24) based on minimum P value from an exact test for HWE (25) and the stratified test for HWE (ref. 26; \( P < 0.001 \)). SNPs with call rates less than 95%; or SNPs with minor allele frequency (MAF) less than 5% were removed from the analysis (27, 28). As a result, 1,348,798 SNPs that passed these quality control measures were included in the GWA analyses of SNP versus IC\textsubscript{50} and SNP versus expression.

**SNP selection and genotyping in lung cancer patients**

SNP selection was based on P values obtained during the analysis of genome-wide SNP versus IC\textsubscript{50} and SNP versus expression, as well as expression versus IC\textsubscript{50} by using LCL data. We selected all SNPs with \( P < 10^{-3} \) from the SNP versus IC\textsubscript{50} analysis. In addition, we defined a SNP peak as a locus that contained at least 2 SNPs associated with cisplatin IC\textsubscript{50} with \( P < 10^{-4} \). SNPs within the SNP peaks were used to carry out an association study with 54,613 expression probesets to identify SNPs that were associated with gene expression (\( P < 10^{-4} \)). Finally an association study was conducted with gene expression and cisplatin IC\textsubscript{50} to identify SNPs that might be associated with cisplatin IC\textsubscript{50} through an influence on gene expression. Therefore, we also selected SNPs with SNP versus expression: \( P < 10^{-4} \) and expression versus IC\textsubscript{50}; \( P < 10^{-4} \) to genotype the patient samples (Supplementary Table S1).

A total of 168 top hit SNPs were selected and genotyped in 1,183 lung cancer patients who received platinum-based chemotherapy. Genotyping was done in the Mayo Clinic Genomics Shared Resource by using a custom-designed Illumina GoldenGate panel. Quality control tests of the genotyping results were done by accounting concordance among 3 control DNA samples that were present in duplicate. SNP call rates, sample call rates, MAFs of SNPs or departure of SNP genotypes from HWE. SNPs were excluded if they failed genotyping or displayed ambiguous clustering, monomorphic genotyping, MAFs of less than 0.01, or significant departures from HWE (\( P < 0.001 \)). SNPs having call rates more than 95% but which passed all other quality control tests were included in the analyses. Of the SNPs with genotyping data, 157 SNPs passed the quality control tests and were included in the analyses.

**Functional validation by siRNA knockdown**

Human NSCLC cell lines, H1437 and H1299 and the SCLC cell line, H196, were used in siRNA screening studies. siRNAs for the candidate genes and nontargeting negative control siRNA pool were purchased from Dharmacon. Specifically, approximately 3,000 to 4,000 cells were seeded into 96-well plates, mixed with an siRNA mixture consisting of 25 nmol/L of specific or negative control siRNAs and the Lipofectamine RNAiMAX reagent (Invitrogen). Twenty-four hours after transfection, the cells were treated with vehicle or increasing concentrations of cisplatin for an additional 72 hours, followed by MTS assay by using the CellTiter 96@ Aqueous Non-Radioactive Cell Proliferation Assay.

**Knockdown efficiency determination by real-time RT-PCR and Western blot analysis**

Total RNA was isolated from cultured cells transfected with controls or specific siRNAs with the Mini RNA isolation kit (ZYMOTHERMO), followed by quantitative reverse transcriptase PCR (qRT-PCR) conducted with the 1-step, Brilliant SYBR Green qRT-PCR master mix kit (Stratagene). Specifically, primers purchased from QIAGEN were used to conducted qRT-PCR by using the ABI StepOne Real-Time PCR System (Applied Biosystems). Western blots were done for DAPK3, METTL6, and RUFY1 by using goat polyclonal antibodies purchased from Santa Cruz biotechnology, Inc. All experiments were conducted in triplicate with \( \beta \)-actin as an internal control.

**Statistical analysis**

A detailed description for GWAS of the LCLs has been described elsewhere (21–23). Briefly, the cisplatin cytotoxicity IC\textsubscript{50} phenotype was calculated for each cell line on the basis of a logistic dose–response model by using the R package “drc” (http://cran.r-project.org/web/packages/drc.pdf). Log-transformed IC\textsubscript{50} values were then compared between genders, batches of samples on the basis of time since purchase and between the CA race and all other samples by using independent samples t tests. An overall comparison of transformed IC\textsubscript{50} values among ethnic groups was done by using an F test on the basis of ANOVA. Because we used LCLs from multiple races/ethnic groups, population stratification was adjusted by using the method developed by Price and colleagues (29), which uses an eigen analysis for detecting and adjusting SNPs. The log-transformed IC\textsubscript{50} values and GCRMA-normalized expression data were adjusted for race by using 5 eigen vectors. The GWA analysis of the association of SNP versus IC\textsubscript{50} or SNP versus expression was done with Pearson correlations by using adjusted SNP, IC\textsubscript{50}, and expression values for each individual. For the siRNA knockdown experiments, group mean values of IC\textsubscript{50} were compared by using Student’s t test.

The OS time was used as the primary endpoint, defined as the time from lung cancer diagnosis to either death or the last known date alive. Patients known to be alive were censored at the time of last contact. All patients included in the analyses were Caucasians. To test for the effect of SNP on OS, we used the Cox regression model that included the effects of SNP genotype dosage (count of minor alleles). A total of 157 SNPs were included in this analysis, and the association was done for NSCLC and SCLC separately because of significant differences between the 2 diseases. To correct for multiple testing of the 157 SNPs assayed in the initial experiment, the Bonferroni corrected \( P \) value threshold of 0.0003 was used to determine statistically significant associations. To
determine whether associations with SNPs should be adjusted for the clinical covariates of age at diagnosis, gender, smoking status, disease stage, and treatment, backward selection was done. The disease stage was included in the final multivariate Cox regression model because it was significantly associated with OS of the lung cancer patients. The disease stage was divided into 5 categories: SCLC with limited versus extensive stages; NSCLC with stages I and II, versus III, versus IV. We used 0.05 as a cutoff for \( P \) values (not adjusted for multiple testing) to select SNPs/genes for further functional validation.

Results

**Correlation analysis of genome-wide SNP versus cisplatin IC\(_{50}\) in LCLs**

Cytotoxicity assays were done for cisplatin to determine the range of variation in drug response, and IC\(_{50}\) value was used as a phenotype to indicate the drug sensitivity for each cell line. The unadjusted average IC\(_{50}\) value for cisplatin in these 283 cell lines was 1.79 ± 1.64 (mean ± SD) \( \mu \)mol/L. Gender had no significant effect on IC\(_{50}\) values \((P = 0.15)\). Caucasian-Americans (CA) were more sensitive to cisplatin compared with the other 2 races (AAs and HCA; \( P = 0.0001 \)). Time since the Coriell Institute acquired the cell lines had no significant effect on cisplatin cytotoxicity \((P = 0.64)\).

A total of 1,348,789 SNPS were used in the genome-wide SNP analysis for the 283 cell lines. Figure 1A depicts the association results graphically. A total of 364 SNPs were associated with cisplatin IC\(_{50}\) with \( P < 10^{-4} \) and 50 SNPs had \( P < 10^{-5} \). None of the top 50 SNPs were within the coding region of a gene. Twenty-three, 16, and 11 SNPs were in introns, 5'-upstream or 3'-downstream flanking regions of genes, respectively. The top 3 SNPs from the GWAS were rs6633130 \((P = 1.66 \times 10^{-7})\), rs6946197 \((P = 1.99 \times 10^{-7})\), and rs12304656 \((P = 3.64 \times 10^{-7})\) at loci containing the PPEF1 (introns), LOC100128030 (3'-downstream) and STYK1 (introns) genes, respectively.

**Survival analysis for the selected SNPs and lung cancer patient samples**

A total of 168 SNPs were selected and genotyped to determine whether any of those SNPs might be associated with patient OS. Table 1 lists basic demographic and clinic data with regard to the patients studied. Of these patients, 656 (55.5%) were male and 527 (44.5%) were female, with a median age of 63.0 years. Most patients had a history of smoking, with 47.3% (\( n = 560 \)) being former smokers and 36.5% \((n = 431)\) current smokers. A total of 550 (46.5%) tumors were classified as adenocarcinoma, 194 (16.4%) as squamous cell carcinoma, and 222 (18.8%) as small cell carcinoma. A total of 881 (74.5%) patients received platinum drugs in combination with surgery and/or radiation and 302 (25.5%) patients received only platinum drugs.

Only disease stage was included in the analysis as a confounder among all the others tested, including age at diagnosis, gender, and smoking status. Nine and 10 different SNPs were associated with OS for NSCLC and SCLC patients, respectively \((P < 0.05); \) Table 2 and Supplementary Fig. S1). However none of the SNPs were statistically significant after correcting for multiple testing. The most significant SNPs, rs1287276 (located within an
with higher IC\textsubscript{50} values in LCLs, and the cells carrying these SNPs would be predicted to be more resistant to cisplatin. Therefore, 12 of the 19 SNPs showed concordant association directions between the 2 results (Table 2) when we compared the association direction of SNPs with clinical OS with that of the same SNP with cisplatin IC\textsubscript{50} values in LCLs.

**Functional characterization of candidate gene**

Although none of the SNPs studied in the patient samples reached statistical significance after multiple testing, to further pursue the possibility that the locus harboring these SNPs might include functional genes, we took advantage of the expression data we had for the cell lines (21–23) to help interpret SNP function and guide the selection of genes for siRNA screening. The overall selection strategy is depicted in Figure 1B. First, we focused on genes located within 200 kb up or downstream of the 12 SNPs which showed a concordant association with the IC\textsubscript{50} phenotype in LCLs and the OS phenotype in patients. A total of 39 genes were found to be located within 200 kb up or downstream of these SNPs, and 7 of the 39 genes close to 4 SNPs had expression levels greater than 50 in LCLs after GCRMA normalization (Fig. 1B). Next, because SNPs might influence cisplatin response through the regulation of gene expression by either cis- or trans-regulation, we also carried out association analysis by using the genotyping data for these 12 SNPs and basal expression array data (54,613 expression probesets) available for all of the LCLs. Eight of 12 SNPs were associated with the expression of 212 probesets (154 unique genes) using a cutoff \( P < 10^{-4} \), and 4 of 12 SNPs were associated with mRNA expression levels of 54 genes with \( P < 10^{-5} \) (Fig. 1B). No cis-regulation was identified. We selected these 54 genes, plus 2 genes with multiple probesets associated with the SNPs to check expression levels in LCLs. However, only 8 of these genes had expression levels more than 50 after GCRMA normalization. Finally, 15 genes, including 7 genes located within 200 kb up or downstream of 4 SNPs (rs11169748, rs2440915, rs5952066, and rs7620841) and 8 genes for which expression was correlated with 3 SNPs (rs11169748, rs2440915, and rs5952066) were selected for siRNA screening (Fig. 1B).

We conducted knockdown experiments for these 15 genes in the NSCLC cell lines H1437 and H1299 and in the SCLC cell line H1496. Knockdown of DAPK3 and METTL6 significantly decreased cisplatin sensitivity in all 3 lung cancer cell lines, whereas knockdown of RUFY1 did not significantly alter cisplatin cytotoxicity (Fig. 2). The rs11169748 and rs2440915 SNPs that were associated with lung cancer OS were also correlated with expression of DAPK3 and METTL6 in LCLs (Fig. 3). However, only DAPK3 expression level was associated with cisplatin IC\textsubscript{50} \( (P = 0.03) \) in LCLs, whereas METTL6 was not \( (P = 0.32) \). To obtain ungenotyped SNPs, we imputed SNPs that were not on
our genotyping platform and that were located within 200 kb up or downstream of these 2 SNPs (rs11169748 and rs2440915), using MACH 1.0 (30), with 1000 Genome Project data (31) as the reference panel. We observed no stronger association with cisplatin IC50 for the imputed SNPs, compared with the observed SNPs (Fig. 3E and F).

Discussion

In this study, we took advantage of the extensive genomic data that we have already obtained for 283 human LCLs, together with DNA samples obtained from lung cancer patients treated with platinum-based therapy to determine whether SNPs identified during a GWAS conducted with LCLs might contribute to our understanding of the drug actions of platinum compounds and the response to platinum compounds in the treatment of lung cancer. Therefore, we first conducted a GWAS for cisplatin in 283 LCLs to identify top candidate SNPs that were associated with platinum cytotoxicity in vitro (Fig. 1 and Supplementary Table S1). We then conducted an association study with selected top candidate SNPs by using DNA samples obtained from NSCLC and SCLC patients (Table 2), followed by functional validation of candidate genes by using human lung cancer cell lines (Fig. 2 and Table 3). We found that 19 SNPs were associated with OS with \( P < 0.05 \) for either NSCLC or SCLC patients (Table 2) and that knockdown of DAPK3 and METTL6, for which expression levels were correlated with the rs11169748 and rs2440915 SNPs (Fig. 3), significantly decreased cisplatin sensitivity in the lung cancer cell lines H1437, H1299, and H196 (Fig. 2).

Previous candidate gene and candidate pathway–based pharmacogenomic analyses have shown that genetic variation may play a role in determining response to platinum-based chemotherapy. Much of the focus has been on SNPs within the DNA damage and repair pathway (2–4, 11–17, 32). However, those results remain controversial. For example, Zhou and colleagues reported that the ERCC1 8092C>A variant allele was associated with a 50% increased risk of death in stage III (A+B) and IV NSCLC patients (32), whereas Wu and colleagues observed that the variant A allele was significantly associated with a shorter OS (HR = 0.68, 95% CI: 0.48–0.95; ref. 3). These previous studies indicate that the effects of each individual SNP are modest and suggest that additional SNPs/genes and/or

### Table 2. The 19 SNPs with the lowest unadjusted \( P \) values that were associated with the OS of lung cancer patients treated with platinum compounds

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>SNP position</th>
<th>MAF</th>
<th>( R^a )</th>
<th>( P )</th>
<th>MAF</th>
<th>HR (95% CI)</th>
<th>( P )</th>
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<td>rs1287276</td>
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<td>63083458</td>
<td>0.242</td>
<td>-0.247</td>
<td>4.72E-05</td>
<td>0.095</td>
<td>1.36 (1.15–1.61)</td>
<td>0.0004</td>
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<td>rs9405302c</td>
<td>5</td>
<td>8228049</td>
<td>0.394</td>
<td>0.267</td>
<td>1.08E-05</td>
<td>0.211</td>
<td>1.22 (1.08–1.37)</td>
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<td>17</td>
<td>379240</td>
<td>0.152</td>
<td>0.251</td>
<td>3.61E-05</td>
<td>0.187</td>
<td>0.82 (0.71–0.94)</td>
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<td>0.126</td>
<td>1.17 (1.05–1.31)</td>
<td>0.006</td>
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<td>111540668</td>
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<td>0.249</td>
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<td>0.396</td>
<td>0.87 (0.78–0.96)</td>
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<td>0.249</td>
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<td>0.029</td>
<td>1.41 (1.08–1.83)</td>
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<td>0.242</td>
<td>6.92E-05</td>
<td>0.200</td>
<td>1.14 (1.03–1.26)</td>
<td>0.014</td>
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<td>rs901893c</td>
<td>1</td>
<td>8228049</td>
<td>0.353</td>
<td>0.239</td>
<td>8.53E-05</td>
<td>0.337</td>
<td>1.14 (1.02–1.27)</td>
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<td>0.237</td>
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<td>0.011</td>
<td>1.75 (1.03–2.97)</td>
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<td>1.80 (1.12–2.89)</td>
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<td>0.310</td>
<td>1.23 (1.00–1.52)</td>
<td>0.050</td>
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</table>

\( ^a \)R values represent correlation coefficients for the associations between SNPs and cisplatin IC50.

\( ^b \)If HR > 1, patients carrying the minor allele had worse OS.

\( ^c \)SNP showed a concordant association with the IC50 and OS phenotypes.
other possible genetic mechanisms such as CpG methylation or copy number variation might influence response to platinum-based therapy.

With advances in genotyping and gene expression technologies, several studies have taken an unbiased approach toward understanding genetic factors influencing response to platinum-based chemotherapy. In LCLs obtained from pedigrees of Centr d’Etudes du polymorphism Human (CEPH) individuals of European background, it was estimated that 30% to 40% of the variation in cisplatin-induced cytotoxicity was because of heritable factors (33, 34). By using this cell line model system, those investigators subsequently identified several genetic variants that contributed to cisplatin and carboplatin cytotoxicity (24, 34–36). However, the results of those studies have not been replicated in cancer
patients treated with platinum-based chemotherapeutics. Our genome-wide pharmacogenomics analysis did not confirm those variants associated with response to cisplatin (24). The difference could be due to differences between populations from which these LCLs are derived. In Huang’s study, the LCLs derived from CEU, European descent and YRI, African descent, whereas our LCLs derived from CA, AA, and HCA. It could also be because...
of confounding factors associated with the use of LCLs, such as cell growth rate and ATP levels (37). Therefore, we carried out siRNA screening for selected candidate genes to pursue the underlying biology. Several previous studies have suggested an important contribution of trans-acting variants to individual variation in human gene expression (38, 39). Among the genes tested, knockdown of DAPK3 and METTL6, for which expression levels correlated with the rs11169748 and rs2440915 SNPs, significantly decreased cisplatin sensitivity in 3 lung cancer cell lines, although METTL6 expression levels were not significantly associated with cisplatin IC50 in LCLs, suggesting a potential difference between LCLs and lung cancer cell lines.

DAPK3, also known as zipper interacting protein kinase (ZIPK) or DAPK-like kinase (DLK), is a member of the DAPK family. DAPK was initially identified as being encoded by a gene whose reduced expression, mediated by antisense cDNA transfection, protected HeLa cells from gamma interferon-induced cell death. DAPK3 contains several putative nuclear localization signal sequences and a leucine zipper domain, required for homo-oligomerization, interaction with other leucine zipper-containing proteins, and for its death-promoting effects (40, 41). DAPK3 has been shown to play distinct roles in tumor suppression and the regulation of apoptosis (42–44). We have shown that knockdown of DAPK3 significantly decreased cisplatin sensitivity in lung cancer cell lines and might be related to OS of NSCLC patients treated with platinum-based therapy.

METTL6 belongs to the methyltransferase superfamily. It has been suggested that chemoresistance and toxicity associated with platinum compound treatment might be mediated through methylation-dependent gene silencing. For example, methylation of FANC, a gene associated with Fanconi anemia, confers cisplatin sensitivity in ovarian cancer cell lines (45). In addition, Ramirez and colleagues observed that 14-3-3s, a cell-cycle checkpoint gene, is methylated in one-third of NSCLC patients and that methylation is related to better median OS for these patients (46). It is possible that METTL6 might influence DNA methylation and further influence response to platinum-based therapy.

In summary, we have identified several genetic variants that are potentially associated with the efficacy of platinum-based chemotherapy in patients with lung cancer by using genome-wide interrogation of LCLs together with genotyping studies by using DNA samples from lung cancer patients, followed by functional validation in lung cancer cells. Our results provide important insight into novel genes and mechanisms that may contribute to variation in response to platinum drug therapy. Most of our patients with advanced NSCLC were treated with cisplatin combination therapy, often with the addition of other chemotherapeutics, radiation, or surgery. Even though the SNPs that we tested were selected on the

### Table 3. Fifteen candidate genes selected for functional validation

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Basis for selection</th>
<th>Distance from the SNP (&lt; 200 kb)</th>
<th>Expression associated with the SNP in LCLs</th>
<th>SNP versus expression in LCLs</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAZAP2</td>
<td>rs11169748</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>TFCP2</td>
<td>rs11169748</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LETMD1</td>
<td>rs11169748</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>GALNT6</td>
<td>rs11169748</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>NA</td>
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<tr>
<td>DAPK3</td>
<td>rs11169748</td>
<td>Yes</td>
<td>0.293</td>
<td>1.05E-06</td>
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<tr>
<td>EML3</td>
<td>rs11169748</td>
<td>Yes</td>
<td>0.310</td>
<td>2.22E-07</td>
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<tr>
<td>LRIG1</td>
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<td>0.273</td>
<td>5.66E-06</td>
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<td>NENF</td>
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<td>Yes</td>
<td>0.292</td>
<td>1.15E-06</td>
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<td>NA</td>
<td>NA</td>
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<tr>
<td>RUFY1</td>
<td>rs11169748</td>
<td>Yes</td>
<td>0.301</td>
<td>4.92E-07</td>
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<tr>
<td>CCDC6</td>
<td>rs2440915</td>
<td>Yes</td>
<td>NA</td>
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<tr>
<td>METTL6</td>
<td>rs2440915</td>
<td>Yes</td>
<td>0.267</td>
<td>9.64E-06</td>
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<tr>
<td>FMR1</td>
<td>rs5952066</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>ELOVL5</td>
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<td>Yes</td>
<td>0.278</td>
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<tr>
<td>METTL9</td>
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<td>Yes</td>
<td>0.250b</td>
<td>3.57E-05</td>
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<td>ETV6</td>
<td>rs7620841</td>
<td>Yes</td>
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<td>NA</td>
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<td>NA</td>
</tr>
</tbody>
</table>

*The gene is located within 200 kb up or downstream of the SNP, and no cis-regulation was identified. NA, no association between the gene and SNP.

*Two probesets were associated with this SNP.
basis of their association with cisplatin cytotoxicity in LCLs, we cannot exclude the possibility of potential interaction of genetic variation with other treatments and their effect on cisplatin treatment outcome in patients with lung cancer. Therefore, it is particularly important that our functional studies confirmed the functional effects of these genes in cisplatin response in vitro. Obviously, additional replication studies in independent sample sets of patients with lung cancer treated with platinum-based chemotherapy would be required to further confirm these findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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