Genetic variants associated with carboplatin-induced cytotoxicity in cell lines derived from Africans

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Abstract

To gain a better understanding of the genetic variants associated with carboplatin-induced cytotoxicity in individuals of African descent, we present a step-wise approach integrating genotypes, gene expression, and sensitivity of HapMap cell lines to carboplatin. Cell lines derived from 30 trios of African descent (YRI) were used to develop a preclinical model to identify genetic variants and gene expression that contribute to carboplatin-induced cytotoxicity. Cytotoxicity was determined as cell growth inhibition at increasing concentrations of carboplatin for 72 h. Gene expression of 89 HapMap YRI cell lines was determined using the Affymetrix GenomeChip Human Exon 1.0 ST Array. Single nucleotide polymorphism genotype and the percent survival at different treatment concentrations along with carboplatin IC50 were linked through whole genome association. A second association test was done between single nucleotide polymorphism genotype and gene expression, and linear regression was then used to capture those genes whose expression correlated to drug sensitivity phenotypes. This approach allows us to identify genetic variants that significantly associate with sensitivity to the cytotoxicity of carboplatin through their effect on gene expression. We found a gene (GPC5) whose expression is important in all carboplatin treatment concentrations as well as many genes unique to either low (e.g., MAPK1) or high (e.g., BRAF, MYC, and BCL2L1) concentrations of drug. Our whole genome approach enables us to evaluate the contribution of genetic and gene expression variation to a wide range of cellular phenotypes. The identification of concentration specific genetic signatures allows for potential integration of pharmacokinetics, pharmacodynamics, and pharmacogenetics in tailoring chemotherapy.

Introduction

Carboplatin, a platinating agent, is commonly used to treat lung, head and neck, and gynecologic cancers (1–3). The drug acts through formation of intrastrand and interstrand cross-links on DNA, which result in DNA strand breaks and lead to cell death (4). Despite its wide usage, carboplatin is associated with severe myelosuppression. The incidence of carboplatin-induced toxicity is highly variable and is associated with drug dosage (4). It has been shown that carboplatin exposure is directly associated with myelosuppression (5) and mucositis (6) in patients.

Using EBV-transformed B-lymphoblastoid cell lines (LCL) derived from healthy individuals within 34 large CEPH pedigrees (northern and western European descent), our laboratory has shown that approximately 17% to 36% of human variation in susceptibility to carboplatin-induced cytotoxicity is due to a genetic component (3). The relationship between carboplatin exposure and toxicity observed in patients (7) led us to hypothesize that different genetic variants may be important at different concentrations of drug.

Several genetic polymorphisms within candidate genes have been shown to correlate with clinical response to carboplatin. For example, XRCC1 R399Q is associated with response to platinum-based neoadjuvant chemotherapy in bulky cervical cancer (8) and in advanced non-small cell lung carcinoma (9). A nonsynonymous single nucleotide polymorphism (SNP), 208G>A (Ala70Thr), in cytidine deaminase is associated with increased incidences of neutropenia when patients were coadministered platinum-containing drugs with gemcitabine compared with individuals treated with gemcitabine alone (10). Lung cancer patients treated with platinum-based therapy carrying the variant allele for C8092A at 3’-untranslated region of ERCC1 have been shown to have a higher incidence of grade 3 or 4 gastrointestinal toxicity (11). In addition, gene expression of GSTM1 (12), MYC (13), ERCC1 (14), BCL2L1 (15), and p53 (16) has also been shown to be related to chemotherapy response in platinum-based cancer treatment.

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3 S. Shukla and M.E. Dolan, unpublished data.
Ethnicity may be an important consideration in studies of the contribution of genetics to treatment outcome for platinating agents. Most of the studies that identified a positive genetic contribution to carboplatin treatment were conducted in the Asian population (8–10). In addition, interethnic differences have been observed in sensitivity to carboplatin in both cell-based (17) and clinical (18, 19) studies. There is a higher mortality rate reported for lung and gynecologic cancer in African Americans compared with Caucasians (20), both of which are commonly treated with platinum-based therapy. For these reasons, a comprehensive study of genetic variants contributing to carboplatin-induced cytotoxicity in individuals of a given population will help us better understand the genetic contribution to clinical response and toxicity.

Recently, our laboratory developed a genome-wide, cell-based approach that integrates genotype, gene expression, and cytotoxicity data to identify potentially functional SNPs associated with chemotherapy-induced cytotoxicity (21, 22). To this end, we chose to apply our model using the International HapMap cell lines, because publicly available dense genotyping allows systemic genome-wide association analysis that would not be possible in other systems. The focus of this study is to identify a definable set of genetic variants and gene expression signatures that contribute to carboplatin sensitivity at different carboplatin concentrations in samples from individuals of African descent. We identify genes important at all concentrations of drug and some unique to either low or high concentrations of drug.

Materials and Methods

Materials

EBV-transformed B-LCLs derived from 30 trios collected from Yoruba in Ibadan, Nigeria (HAPMAPPT03, YRI) were purchased from the Coriell Institute for Medical Research.4 Cell lines were maintained and diluted as described (17). Carboplatin was purchased from Sigma-Aldrich.

Cytotoxicity Assay

Cell growth inhibition was evaluated at concentrations of 0, 10, 20, 40, and 80 μmol/L of carboplatin. Carboplatin was dissolved in water immediately before use. Percent cell survival after 72 h treatment of increasing concentrations of carboplatin was determined using the nontoxic colorimetric-based assay, alamarBlue, as described previously (17). Drug concentration required to inhibit 50% of cell growth (IC50) was obtained through percent survival concentration curve fitting. We determined cell proliferation rate by evaluating cell growth at 72 h for each cell line without drug addition.

Genotype and Cytotoxicity Association Analysis

SNP genotypes of YRI population were downloaded from the International HapMap database5 (Release 22, nonredundant and rs_strand version), and 2,286,186 SNPs with minor allele frequencies >5% and no Mendelian inheritance transmission errors in the YRI trios were used. Percent cell survival at each drug concentration and IC50 values were log2 transformed to obtain approximate normally distributed phenotypes. The quantitative transmission disequilibrium test (QTDT) was done to identify any genotype-cytotoxicity association using QTDT software6 (23) with sex as a covariate. \( P \leq 0.0001 \) was considered statistically significant. The binomial tests were done between the probability of our significant findings and the probability of random discovery.

Genotype and Gene Expression Association Analysis

Baseline gene expression was evaluated in 89 YRI LCLs using Affymetrix GeneChip Human exon 1.0 ST array (Exon Array) as described previously (22). The gene expression data described in this article has been deposited into GEO (GenBank accession no. GSE7761). Significant SNPs generated from the genotype-cytotoxicity association in YRI were tested for their association with gene expression using the QTDT test with gender as a covariate as described previously (22). A Bonferroni correction with the number of gene expressions tested \((P_c < 0.05)\) was used to adjust raw \( P \) values after QTDT analysis.

Linear Regression Analysis of Gene Expression and Sensitivity to Carboplatin

To examine the relationship between gene expression and sensitivity to carboplatin, a general linear model was constructed with log2-transformed carboplatin sensitivity phenotypes as the dependent variable and transformed gene expression level together with an indicator for gender as the independent variables (22). If a SNP was significantly associated with a carboplatin cytotoxicity phenotype and the same SNP was significantly associated with gene expression, then the above approach was used to test whether gene expression significantly predicted that phenotype. Transcript cluster expression, with gender as a covariate in the model, was tested as a predictor of carboplatin sensitivity in the YRI population. \( P < 0.05 \) was considered statistically significant. If a gene whose expression was associated with one or more SNP genotypes and correlated with sensitivity to carboplatin, this gene is defined as a target gene. The linkage disequilibrium of significant SNPs was evaluated using Haplovie version 3.32.7

Enrichment Analysis of Functional Annotation Categories

We use DAVID bioinformatics tools8 to identify enriched functional annotation categories for target genes. Affy_ID (Transcript cluster ID) identifier was used to upload target gene lists generated for each carboplatin phenotype (percent survival at 10, 20, 40, and 80 μmol/L and IC50).

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4 http://www.locus.umdnj.edu/cecr
5 http://www.HapMap.org
6 http://www.sph.umich.edu/csg/abecasis/QTDT
7 http://www.broad.mit.edu/mpg/haplovie/
8 http://david.abcc.ncifcrf.gov/
Entrez_gene_ID identifier was used to upload the background gene list, which contains 10,830 genes that are expressed in LCLs. Three Gene Ontology (GO) terms (biological process, cell component, and molecular function) were evaluated. Uncorrected \( P < 0.05 \) was considered statistically significant.

**Relationship between SNPs Important for Carboplatin and Cell Proliferation Rate**

Cell proliferation rate can be a determinant of sensitivity to chemotherapy. We, therefore, interrogated the relationship between SNP genotype and cell proliferation rate only for those SNPs found to be significantly associated with carboplatin cytotoxic phenotypes (at any concentration) through gene expression. Cell proliferation rate for each LCL was \( \log_{2} \) transformed and 208 significant SNPs were tested for their association with cell proliferation rate using the QTDT test with gender as a covariate. A Bonferroni correction with the number of SNPs tested \( (P < 0.05) \) was used to adjust raw \( P \) values. In addition, to better understand when controlling for the cell proliferation rate, whether SNPs that associated with phenotypes through expression are still important, the QTDT test was done between transformed cytotoxic phenotypes and 208 significant SNP genotypes with cell proliferation rate as a covariate. \( P < 0.05 \) was considered significant.

**Results**

**QTDT Genotype-Cytotoxicity Association**

The median percent cell survival ranges from 67.4\% to 28.7\% following exposure to increasing concentrations of carboplatin (10-80 \( \mu \)mol/L) for 72 h (Table 1; Supplementary Fig. S1).

<table>
<thead>
<tr>
<th>Drug concentration (( \mu )mol/L)</th>
<th>Median (range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>67.4 (49.5-98.6)</td>
</tr>
<tr>
<td>20</td>
<td>55.0 (36.3-90.3)</td>
</tr>
<tr>
<td>40</td>
<td>43.0 (22.7-78.2)</td>
</tr>
<tr>
<td>80</td>
<td>28.7 (12.8-64)</td>
</tr>
</tbody>
</table>

\( *n = 89. \)

The number of SNPs associated with carboplatin cytotoxic phenotypes and also associated with gene expression (Bonferroni corrected with number of transcript clusters; \( P < 0.05 \)) vary among different concentrations and IC\(_{50}\), we found a minimum of 58 SNPs (associated with percent survival after 10 \( \mu \)mol/L carboplatin treatment) and a maximum of 132 SNPs (associated with percent survival after 80 \( \mu \)mol/L carboplatin treatment) met this criteria. The number of significantly associated SNPs and transcript clusters at each phenotype are shown in Table 2.

**Linear Regression of Gene Expression and Carboplatin Sensitivity**

We examined the correlation between gene expression and the five carboplatin sensitivity phenotypes (percent survival after 10, 20, 40, and 80 \( \mu \)mol/L treatment and IC\(_{50}\)) using a general linear model that was constructed to reflect the trio relationship in our samples. The expression of 33 to 71 transcript clusters was found to be significantly correlated to carboplatin cytotoxic phenotypes (percent survival after 10-80 \( \mu \)mol/L carboplatin treatment; \( P < 0.05 \); Table 2). The expression levels of these genes were significantly associated with 39 to 97 SNPs (Table 2).

Interestingly, we identified two SNPs in complete linkage disequilibrium (rs1031324 and rs1993034) whose genotypes are significantly associated with carboplatin cytotoxic phenotypes at all concentrations and IC\(_{50}\) through glypican 5 (GPC5) gene expression (Fig. 1). Other SNPs were associated with cellular survival following lower concentrations of carboplatin treatment (10 and 20 \( \mu \)mol/L). One example was the association between the genotype of rs3821666 (located within an intron of the MEDI2L/PRY14 gene on chromosome 3) and percent survival after 10 \( \mu \)mol/L (Fig. 2A) and 20 \( \mu \)mol/L carboplatin treatment (\( P = 4 \times 10^{-5} \) and \( 1 \times 10^{-4} \), respectively) and with expression of MAPK1 on chromosome 22 (\( P = 2 \times 10^{-6} \); Fig. 2B), whose expression also significantly correlated to percent survival after 10 \( \mu \)mol/L (Fig. 2C) and 20 \( \mu \)mol/L carboplatin treatment (\( P = 1 \times 10^{-2} \) and \( 9 \times 10^{-3} \), respectively). Similarly, there were SNPs only associated with percent survival following higher concentrations of carboplatin treatment. For example, rs11993726 (located in HMBOX1 gene on chromosome 8) is significantly associated with percent survival following treatment with 40 \( \mu \)mol/L (Fig. 3A) and 80 \( \mu \)mol/L carboplatin (\( P = 8 \times 10^{-5} \) and \( 1 \times 10^{-4} \), respectively). This SNP is also
associated with expression of \textit{BRAF} on chromosome 7 \((P = 2 \times 10^{-6}; \text{Fig. 3B})\), which is correlated to these two carboplatin sensitivity phenotypes \((P = 4 \times 10^{-4} \text{ and } 5 \times 10^{-3}, \text{ respectively; Fig. 3C})\). The hierarchical clustering of the four carboplatin cytotoxic phenotypes support that lower concentrations (10 and 20 \mu mol/L) are phenotypically more similar than those at higher concentrations (80 \mu mol/L; Supplementary Fig. S1).9

Genes of interest were also identified at individual concentrations of drug: rs17001543 genotype, \textit{XRCC2} expression, and percent survival after 40 \mu mol/L carboplatin treatment; rs1704644 genotype, \textit{MYC} expression, and percent survival after 80 \mu mol/L carboplatin treatment; and rs187006 (in complete linkage disequilibrium with rs2635266) genotype, \textit{BCL2L1} expression, and percent survival after 80 \mu mol/L carboplatin treatment (Supplementary Figs. S2-S4; Supplementary Table S1).9

**Enrichment Analysis of Functional Annotation Categories**

To better understand the gene classifications whose expression is correlated with carboplatin sensitivity, we evaluated the enrichment of functional annotation categories for these target genes. The enriched GO pathways at each treatment concentration are shown in Table 3. It is clear that different treatment conditions are associated with different enriched GO pathways. Genes related to lymphocyte proliferation process are enriched at 20 \mu mol/L (e.g., \textit{CD3E} and \textit{IL12B}), whereas genes related to apoptosis (e.g., \textit{BCL2L1} and \textit{BRAF}) are enriched after 80 \mu mol/L carboplatin treatment.

**SNP Effects on Cell Proliferation Rate**

Evidence suggest that the mechanism of cell growth inhibition by carboplatin is intra- and inter-DNA cross-links (4) that likely manifests itself to a greater degree in more rapidly growing cells. Our data in LCLs indicated a significant correlation between drug effect as measured by IC\textsubscript{50} and cellular proliferation rate (Supplementary Fig. S5). Therefore, we further interrogated the SNPs identified at each drug concentration to determine if these associations are through an effect on rate of cellular proliferation. Of the 208 significant SNPs associated with at least one carboplatin sensitivity phenotype through gene expression, 50 SNPs were also associated with cellular proliferation rate (Bonferroni correction with number of SNPs tested; \(P < 0.05\)). If raw \(P < 0.05\) was used as significant cutoff, 141 SNPs were associated with cell proliferation rate. QTDT analysis was done between the 208 significant SNPs and their corresponding cytotoxic phenotypes but using cellular proliferation rate as a covariate. One hundred seventy-nine of the 208 SNP genotypes remain significantly associated with carboplatin cytotoxic phenotypes (\(P < 0.05\)); however, using a stringent Bonferroni correction with number of SNPs tested (\(P < 0.05\)), 68 SNPs remain significant (Supplementary Table S1).9

The genotypes of rs1031324 and rs1993034 (associated with \textit{GPC5} expression), which associated with all cytotoxic phenotypes, were also associated with cell proliferation rate (\(P = 4 \times 10^{-4}\)). When including cell proliferation rate as a covariate in a linear regression model, these SNP genotypes are still associated with carboplatin cytotoxicity (\(P = 0.009, 0.009, 0.017, 0.016, \text{ and } 0.003\) for percent survival at 10, 20, 40, and 80 \mu mol/L and IC\textsubscript{50}, respectively). rs3821666 genotype (associated with \textit{MAPK1} expression), which was associated with percent survival at 10 and 20 \mu mol/L, is also associated with cell proliferation (\(P = 8 \times 10^{-4}\)). When controlled for proliferation, it is still associated with percent survival after 10 and 20 \mu mol/L treatment (\(P = 0.005 \text{ and } 0.021\), respectively). In contrast, the rs11993726 genotype (associated with \textit{BRAF} expression and percent survival at 40 and 80 \mu mol/L) is not associated with cell proliferation (\(P > 0.05\)).

**Table 2. Number of significant associations for each concentration of carboplatin**

<table>
<thead>
<tr>
<th>Approach</th>
<th>10 \mu mol/L</th>
<th>20 \mu mol/L</th>
<th>40 \mu mol/L</th>
<th>80 \mu mol/L</th>
<th>IC\textsubscript{50} (\mu mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP associated with phenotype ((P \leq 0.0001))</td>
<td>470 SNPs</td>
<td>535 SNPs</td>
<td>574 SNPs</td>
<td>741 SNPs</td>
<td>439 SNPs</td>
</tr>
<tr>
<td>SNP associated with gene expression</td>
<td>58 SNPs</td>
<td>75 SNPs</td>
<td>66 SNPs</td>
<td>132 SNPs</td>
<td>65 SNPs</td>
</tr>
<tr>
<td>\textbf{(Bonferroni-corrected } P &lt; 0.05)</td>
<td>\textit{(75 transcript clusters)}</td>
<td>\textit{(103 transcript clusters)}</td>
<td>\textit{(85 transcript clusters)}</td>
<td>\textit{(133 transcript clusters)}</td>
<td>\textit{(71 transcript clusters)}</td>
</tr>
<tr>
<td>Gene expression correlated with phenotype ((P &lt; 0.05))</td>
<td>33 Transcript clusters</td>
<td>53 Transcript clusters</td>
<td>37 Transcript clusters</td>
<td>71 Transcript clusters</td>
<td>38 Transcript clusters</td>
</tr>
<tr>
<td>\textit{(39 SNPs)}</td>
<td>\textit{(65 SNPs)}</td>
<td>\textit{(41 SNPs)}</td>
<td>\textit{(97 SNPs)}</td>
<td>\textit{(46 SNPs)}</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Knowledge about genetic susceptibility to carboplatin toxicity could help to determine the optimal treatment schedule and dosage and, in some cases, avoidance of this drug if toxicity risks outweigh potential benefits. Most studies have evaluated candidate genes associated with platinating agents; however, building a whole genome model provides an unbiased approach that could provide leads for clinical testing, ultimately improving the utility of...
these agents. To this end, our laboratory recently developed a genome-wide model that integrates genotype, gene expression, and drug cytotoxicity, referred to as the “triangle approach,” to identify potentially functional pharmacogenetic SNPs associated with chemotherapy-induced cytotoxicity (21, 22). Because a positive correlation has been observed between carboplatin dosage and toxicity (7), we applied the triangle approach at each treatment concentration. We identified 208 genetic variants that were significantly associated with a single or multiple carboplatin-induced cytotoxicity phenotypes (percent survival after 10, 20, 40, and 80 μmol/L carboplatin treatment or IC50) through the expression of 210 genes in cell lines derived from the YRI population. Two of these SNPs are associated with carboplatin cytotoxicity at all carboplatin treatment concentrations, whereas others are associated with either low or high concentrations of the drug.

Two SNPs (rs1031324 and rs1993034 in complete linkage disequilibrium) have genotypes that are significantly associated with carboplatin sensitivity phenotypes at all treatment concentrations and IC50 through GPC5 gene expression. GPC5 is a cell-surface heparin sulfate proteoglycan that is thought to play a role in the control of cell division and growth regulation (24). Genomic amplification of the 13q31-32 region, where GPC5 is located, is reported in many tumors, including rhabdomyosarcoma (25) and mantle cell lymphoma (26). Yu et al. have shown that overexpression of GPC5 may contribute to development and/or progression of lymphomas and other tumors (27). Not surprisingly, we found higher GPC5 expression also correlated to higher cellular proliferation rate, indicating that more rapidly growing cells are more susceptible to carboplatin damage. In addition, we found significant association between rs1031324 (or rs1993034) genotype and GPC5 expression and between these SNPs and the cell proliferation rate (P = 4 × 10⁻⁸). These SNPs and GPC5 are located on different chromosomes, indicating a distant-acting effect. The proportion of false-positive SNP expression relationships may be greater among distant-acting effects (28); however, we have some level of confidence in these SNPs because an association is observed at all drug concentrations. GPC5 is a gene known to be important in cellular proliferation (24); therefore, the association with each drug concentration may simply be a result of the association of these SNPs with cell proliferation. However, when using cell proliferation rate as a covariate, we showed these SNP genotypes (rs1031324 and rs1993034) remain significantly associated with carboplatin cytotoxicity, indicating that they may be acting directly and indirectly (through effect on cell proliferation) on carboplatin-induced cytotoxicity.

Of the 208 SNPs associated with carboplatin cytotoxicity, 50 SNPs were associated with cell proliferation rate, indicating that a group of “cytotoxicity-associated” SNPs...
may be intermediary SNPs. Growth rate has been shown to affect cellular sensitivity to chemotherapeutic drugs (29). Because our long-term goal is to identify patients “at risk” for toxicity or nonresponse using genetic information, SNPs associated either directly or indirectly with drug may still be important to consider. Furthermore, when using cell proliferation rate as a covariate, 179 of 208 SNP genotypes are still associated with carboplatin cytotoxic phenotypes, indicating that the majority of our findings are specific to this drug.

In addition to the SNPs that associated with cytotoxicity at all carboplatin concentrations, we also identified many SNPs and gene expressions that associated with percent survival following treatment with high or low carboplatin concentrations. Itoh et al. have shown that treatment of the squamous cell carcinoma cell line MIT7 with carboplatin resulted in apoptosis in a dose-dependent manner (30). Interestingly, our GO enrichment analysis showed genes related to apoptosis are enriched only after 80 μmol/L carboplatin treatment, whereas genes related to lymphocyte proliferation process are enriched at 20 μmol/L.

Our laboratory identified interethnic differences in cellular sensitivity to carboplatin treatment with YRI being less sensitive than CEU (Caucasian with northern and western European ancestry; ref. 17). Alberts et al. have evaluated 324 ovarian cancer patients who were treated with cyclophosphamide and either carboplatin or cisplatin. They found that White patients survived significantly longer than Black patients regardless of age, performance status, or stage of disease (18). Clearly, more research systematically studying interethnic differences in sensitivity to chemotherapeutic drugs is needed. The model used here can provide population-specific genetic variants and potentially unveil the genetic variants that contribute to the interethnic differences in response to carboplatin.

Candidate gene studies have identified several genetic polymorphisms associated with patient response following platinum therapy, including 208G>A SNP in cytidine deaminase (10), R399Q SNP in XRCC1 (8, 9), and C8092A SNP in ERCC1 (11). These three polymorphisms did not come up in our model. Some explanations exist: (a) our population was of African descent, whereas the clinical trials were done in either Asians or Caucasians; (b) the clinical trials included multiple drugs; and (c) SNPs of interest may not be interrogated in HapMap samples or the minor allele frequency of the SNP is less than 5% in the YRI samples, thereby failing to meet our inclusion evaluation criteria; or (d) our model in LCLs may not reflect tumor genetics or gene expression in tissues of toxicity. For example, a nonsynonymous SNP, 208G>A (Ala70Thr)

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Figure 2. Relationship between rs3821666, MAPK1 gene expression, and percent survival after 10 μmol/L carboplatin treatment in the YRI samples. A, rs3821666 genotype and log2-transformed percent survival after 10 μmol/L carboplatin treatment association. B, rs3821666 genotype and log2-transformed MAPK1 expression association. C, log2-transformed MAPK1 expression and log2-transformed percent survival after 10 μmol/L carboplatin treatment correlation.
in cytidine deaminase was reported to associate with increased incidence of neutropenia in patients coadministered platinum-containing drugs with gemcitabine compared with gemcitabine alone (10). Although this particular SNP was identified in the Japanese population with allele frequency of 0.037 (10), in the most recent HapMap database, this SNP is not reported in any population. Another example is the A allele carriers of rs25487 (also referred to XRCC1 R399Q) have lower response rate to platinum-based therapy (8, 9). The allele frequencies were 0.274, 0.279, and 0.1 in Chinese (CHB), Japanese (JPT), and YRI, respectively. One possible reason we did not observe any significant association between this SNP and carboplatin cytotoxicity phenotype may be a result of the lower rare allele frequency in the YRI samples. Lastly, Suk et al. reported A allele carriers of rs3212986 (also referred to ERCC1 C8092A) treated with platinating agents have a higher incidence of severe gastrointestinal toxicity but no association with hematologic or overall toxicity (11). LCLs may be a better model for prediction of hematologic toxicity; thus, our lack of association between rs3212986 genotype and carboplatin cytotoxicity is in agreement with this clinical data.

In addition to genetic variants, expression variation of GSTM1 (12), MYC (13), ERCC1 (14), BCL2L1 (15), and p53 (16) have also been shown to be related to chemoresistance in platinum-based cancer treatment. Our data confirmed the role of both MYC and BCL2L1. For example, we found an inverse correlation between MYC expression and carboplatin cytotoxicity (percent survival after 80 µmol/L treatment and IC50), in agreement with Iba et al.’s clinical findings that epithelial ovarian cancer patients with higher MYC tumor expression had better 5-year survival following platinum-based chemotherapy (13). BCL2L1 expression has been shown to confer resistance to carboplatin in oral squamous cell carcinoma (15, 31) and prostate cancer cells (32). Although there were less significant correlations between the expression of p53 or GSTM1 and carboplatin cytotoxicity (P = 0.005-0.04 for percent survival at various concentrations), we did not find SNP genotypes associated with cytotoxicity and expression of these genes. Lastly, ERCC1 is expressed in LCLs, but there was not a significant correlation between the baseline expression of ERCC1 and carboplatin-induced cytotoxicity.

In addition to confirm correlations between MYC and BCL2L1 expression with carboplatin-induced cytotoxicity, we identified the potential genetic regulators that are associated with these genes. We found rs17074644 genotype is associated with MYC expression; similarly, rs187006 genotype is associated with BCL2L1 expression. Both SNPs are associated with survival at 80 µmol/L drug. Interestingly, baseline expression of either gene is correlated with percent survival after 20, 40, and 80 µmol/L carboplatin treatment (data not shown). Given the

Figure 3. Relationship between rs11993726, BRAF gene expression, and percent survival after 40 µmol/L carboplatin treatment in the YRI samples. A, rs11993726 genotype and log2-transformed percent survival after 40 µmol/L carboplatin treatment association. B, rs11993726 genotype and log2-transformed BRAF expression association. C, log2-transformed BRAF expression and log2-transformed percent survival after 40 µmol/L carboplatin treatment correlation.

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literature support that platinum agents can inhibit MYC expression (33, 34), the possibility exists that treatment with higher concentrations of carboplatin inhibits MYC expression to a greater degree than at lower concentrations, thereby explaining why the SNP genotype effect is significantly associated with higher treatment concentrations, not lower concentrations.

Although the full implications and biological significance of all genes and networks identified through our approach are not yet fully understood, our studies should direct both functional follow-up studies and clinical studies by providing a strong list of candidate genes. They may serve as a platform to further explore relevant mechanisms and improve our understanding of the molecular basis of carboplatin-induced cytotoxicity. Evaluating the candidate SNPs and gene expression in our model indicates that the LCL model is a reasonable screening tool to identify previously known and unknown genetic polymorphisms and expression signatures for carboplatin-induced cytotoxicity. One should keep in mind that some rare genetic polymorphisms may not be captured in HapMap samples and there are likely expression and post-translational modification differences in various tissues; therefore, genes not expressed in LCLs may still be important cytotoxicity predictors in other tissues. In addition, genetic predictors were generated in cell lines derived from individuals collected in a community in Ibadan, Nigeria. These samples do not necessarily represent all Yoruba people, Africans, or African Americans (a complex admixture). Further validation is warranted in extending our findings into an African American population.

Disclosure of Potential Conflicts of Interest

Dr. Dolan has presented seminars at Affymetrix funded meetings. The other authors reported no potential conflicts of interest.

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Genes Contributing to Carboplatin Toxicity


Molecular Cancer Therapeutics

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R. Stephanie Huang, Shiwei Duan, Emily O. Kistner, et al.


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