Functional Genomics Reveals Diverse Cellular Processes That Modulate Tumor Cell Response to Oxaliplatin

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Abstract

Oxaliplatin is widely used to treat colorectal cancer, as both adjuvant therapy for resected disease and palliative treatment of metastatic disease. However, a significant number of patients experience serious side effects, including prolonged neurotoxicity, from oxaliplatin treatment creating an urgent need for biomarkers of oxaliplatin response or resistance to direct therapy to those most likely to benefit. As a first step to improve selection of patients for oxaliplatin-based chemotherapy, we have conducted an in vitro cell-based small interfering RNA (siRNA) screen of 500 genes aimed at identifying genes whose loss of expression alters tumor cell response to oxaliplatin. The siRNA screen identified twenty-seven genes, which when silenced, significantly altered colon tumor cell line sensitivity to oxaliplatin. Silencing of a group of putative resistance genes increased the extent of oxaliplatin-mediated DNA damage and inhibited cell-cycle progression in oxaliplatin-treated cells. The activity of several signaling nodes, including AKT1 and MEK1, was also altered. We used cDNA transfection to overexpress two genes (LTBR and TMEM30A) that were identified in the siRNA screen as mediators of oxaliplatin sensitivity. In both instances, overexpression conferred resistance to oxaliplatin. In summary, this study identified numerous putative predictive biomarkers of response to oxaliplatin that should be studied further in patient specimens for potential clinical application. Diverse gene networks seem to influence tumor survival in response to DNA damage by oxaliplatin. Finally, those genes whose loss of expression (or function) is related to oxaliplatin sensitivity may be promising therapeutic targets to increase patient response to oxaliplatin. Mol Cancer Res; 9(2); 173–82. © 2010 AACR.

Introduction

Oxaliplatin is a third-generation, platinum-based chemotherapeutic agent that has significant activity in colorectal carcinoma (CRC). Adjuvant therapy with oxaliplatin, combined with fluoropyrimidine-based (5-FU) chemotherapy, results in significant increases in disease-free survival rates in patients with stage II/III colon cancer (1). In the metastatic setting, combination therapy with 5-FU and oxaliplatin is the most commonly used frontline regimen, with superior response rates and longer survival than with 5-FU alone (2, 3). However, it is apparent that not all patients benefit from oxaliplatin treatment, and in the face of significant side effects associated with oxaliplatin, most notably prolonged neurotoxicity, there is a great need for clinical tools to guide the use of oxaliplatin in those patients who are most likely to derive benefit from it.

Oxaliplatin induces cytotoxicity through the formation of platinum–DNA adducts, which, in turn, activate multiple signaling pathways (4). Alterations in drug efflux and uptake, DNA repair, and inactivation of the apoptosis pathways have been hypothesized to promote resistance to platinum agents such as carboplatin and cisplatin (5, 6). None of these putative markers of oxaliplatin sensitivity and resistance have been clinically validated, and, at present, there are no markers established in clinical use for selecting CRC patients for oxaliplatin therapy.

The current clinical practice used for making CRC treatment decisions is determined by clinical and pathologic staging. However, these prognostic tools do not predict drug response in an individual patient. Recent insights into the genomics of cancers have enabled development of diagnostic tests that inform clinical decisions for cancer patients (7–10). To further advance the personalization of CRC treatment, there is a need for a greater understanding of the genetic alterations in CRC tumors that are associated with patient sensitivity or resistance to oxaliplatin. One approach to identifying genes responsible for drug sensitivity or resistance is the use of in vitro loss-of-function (LOF) genetic screens via RNA-mediated interference (RNAi). Genes that modulate oxaliplatin sensitivity in vitro can be taken forward for testing in clinical studies and, ultimately, may serve to be...
clinically useful predictive biomarkers to guide patient selection for therapy with oxaliplatin.

We carried out a small interfering RNA (siRNA) screen in human CRC cell lines to identify genes whose LOF modulates in vitro tumor cell response to oxaliplatin. Our primary screen targeted 500 genes involved in DNA repair, drug transport, metabolism, apoptosis, and regulation of the cell cycle, and utilized 4 unique siRNA duplexes over 7 different oxaliplatin concentrations per gene. Here, we report 27 genes whose loss of expression can significantly alter the response (through either increased sensitivity or increased resistance) of colon tumor cell lines to oxaliplatin. These results provide a foundation for future work in clinical samples from CRC patients treated with oxaliplatin and also reveal insights into the heterogeneity of cellular processes regulating tumor cell response to DNA damage.

Materials and Methods

Cell lines
Colon cancer cell lines HCT 116 (ATCC# CCL-247) and SW480 (ATCC# CCL-228) were obtained from the American Type Culture Collection and were maintained in McCoy's 5A medium supplemented with 10% FBS, 1.5 mmol/L l-glutamine, and 1% Antibiotic-Antimycotic (Invitrogen).

siRNA screening and drug treatments
Four siRNA sequences for each targeted gene were picked from the Whole Human Genome siRNA Library (Qiagen) to create 6 custom 384-well assay plates. All assay plates included negative control siRNAs (Non-Silencing, All-Star Non-Silencing, and GFP), and 2 positive control siRNAs for transfection (UBBsi1 and All-Star Cell Death Control), all of which were purchased from Qiagen. Selected siRNAs were printed individually into white solid 384-well plates (1 μL of 0.667 μmol/L siRNA per well for a total of 9 ng of siRNA), using a Biomek FX (Beckman Coulter). Lipofectamine 2000 (Invitrogen) was diluted in serum-free McCoy's 5A media and 20 μL was transferred into each well of the 384-well plate containing siRNAs (final ratio of 7.4 nL of lipid per ng of siRNA). After an incubation period of 30 minutes at room temperature to allow the siRNA and lipid to form complexes, 20 μL of HCT 116 cells (2.5 × 10^5 cells/mL) in antibiotic-free McCoy's 5A media were added into each well. Transfected cells were incubated for 24 hours prior to the addition of 10 μL per well of different concentrations of oxaliplatin (35.0, 3.75, 3.0, 2.0, and 1.5 μmol/L) and vehicle control (DMSO) for a total assay volume of 50 μL. Oxaliplatin was obtained from Sigma. Cell viability was measured 72 hours post-drug treatment, using the CellTiter-Glo assay (Promega), measured on an Analyst GT Multimode reader (Molecular Devices). A replicate of the screen was also done, resulting in a total of 56 data points per gene. Cell viability data were normalized to the median value of negative control siRNAs, and IC₅₀ values were calculated using Prism 5.0 (GraphPad).

Hit identification and statistical analysis
The effect of siRNA treatment on the IC₅₀ of oxaliplatin was expressed as the log₂ fold shift of the median IC₅₀ of siRNA-treated cells relative to the median IC₅₀ of non-silencing siRNA control-treated cells. Hits were identified as those with a median IC₅₀ shift of median IC₅₀ ± 3 MAD (median absolute deviations) or greater (11, 12). To assign statistical significance to siRNA hits identified from the siRNA screen, we then modeled the collective activities of the 4 individual siRNAs used for each gene by using redundant siRNA activity (RSA) analysis (13). Briefly, the normalized, log₂ transformed IC₅₀ shifts of all siRNAs were ranked ordered and the rank distribution of all siRNAs targeting the same gene was examined and a P value was calculated on the basis of an iterative hypergeometric distribution formula (13). siRNAs with P < 0.05 values were considered as significant. Subsequently, only genes with a median IC₅₀ shift of median IC₅₀ ± 3 MAD or greater and an RSA P < 0.05 value were considered robust hits and analyzed further. All other tests of significance were 2-sided, and P < 0.05 values were considered significant.

Validation siRNA knockdown
For validation of siRNA hits, ON-TARGETplus siRNAs (Thermo Scientific), containing pools of 4 siRNAs per gene, were utilized (Supplementary Table S3). Seventy microliters of HCT 116 or SW480 cells (1.0 × 10⁵ cells/mL) was plated in black, clear-bottomed 96-well plates in antibiotic-free McCoy’s 5A medium and allowed to adhere overnight. Cells were then transfected with 25 nmol/L of siRNA by using DharmaFECT transfection reagent (Thermo Scientific). Following a 4-hour incubation period, 10 μL per well of an 11-point, 2-fold serial dilution of oxaliplatin (50 μmol/L maximum concentration) was then added, for a total assay volume of 100 μL. Assays were done in triplicate, with ON-TARGETplus Non-Targeting siRNA (Thermo Scientific) as a negative control, with biological replicates. Cell viability was measured 72 hours later using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega), and IC₅₀ values calculated using Prism 5.0 (GraphPad). siRNA knockdown after 72 hours was validated by qRT-PCR, using the Roche LightCycler480 (Supplementary Fig. S3A).

Overexpression experiments
Full-length LTBR or TMEM30A ORFs were cloned into pCMV-ML4 or pCMV-ML5, respectively (Origene), and validated by sequencing. Transfection was carried out using Turbofectin 8.0 (Origene) in 96-well format as per manufacturer’s instructions, using 100 ng of cDNA per well. Following a 4-hour incubation period, 10 μL per well of an 11-point, 2-fold serial dilution of oxaliplatin (50 μmol/L maximum concentration) was then added. Assays were done in triplicate, using the empty pCMV-ML4 vector or empty pCMV-ML5 vector as a negative control, with biological replicates. Cell viability was measured 0, 24, 48, and 72 hours later (Fig. 2C and Supplementary Fig. S3C) by using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega), and IC₅₀ values calculated using Prism 5.0.
DNA damage, phosphorylation, and caspase-3 cleavage assays

DNA damage was assessed by quantification of apurinic/apyrimidinic (AP) sites (BioVision) following manufacturer’s instructions.

Phosphorylation status of Akt1 (Ser473), MAP/ERK kinase (MEK) 1 (Ser217/221), p38 mitogen activated protein kinase (MAPK; Thr180/Tyr182), STAT3 (Tyr705), and NF-κB p65 (Ser536) was determined using the PathScan Signaling Nodes Multi-Target Sandwich ELISA (Cell Signaling Technology) as per manufacturer’s instructions. The phosphorylation status of p53 (Ser15), Bad (Ser112), PARP (Asp214), and cleavage status of caspase-3 was determined using the PathScan Apoptosis Multi-Target Sandwich ELISA (Cell Signaling Technology), following manufacturer’s instructions. Raw signal intensity was normalized to either total Akt or Bad protein levels. Assays were done in duplicate, and the log2 fold change (OD450 siRNA-malimized to either total Akt or Bad protein levels. Assays were manufacturer’s instructions. Raw signal intensity was normalized to the housekeeping gene, β-actin, and results were expressed as normalized expression relative to empty pCMV-XL4 or empty pCMV-XL5 vector-transfected cells ($e^{-\Delta\Delta C_{\text{P}}}$; Supplementary Fig. S3B).

Results and Discussion

A siRNA screen identifies genes that control tumor cell sensitivity to oxaliplatin

We screened a custom siRNA library targeting 500 genes with putative roles in DNA damage repair, apoptosis, cell-cycle regulation, drug metabolism, and transport, using the colorectal cancer tumor cell line HCT 116 (Supplementary Table S1). The siRNA library contained 4 siRNAs targeting each gene, with each siRNA transfected individually. The screen was conducted in duplicate, with a non-silencing siRNA negative control. siRNAs were used at 17 nmol/L to reduce off-target effects. Twenty-four hours after transfection, 5 different concentrations of oxaliplatin (35.0, 3.75, 3.0, 2.0, and 1.5 μmol/L) and vehicle control (DMSO) were added and cell viability was measured 72 hours after the addition of drug. The mean Z’ factor for the screen was 0.78, suggesting that our assay had a robust signal-to-noise ratio (Supplementary Fig. S1B).

Two stringent criteria were used to limit the discovery of false-positive genes. First, we identified all genes whose silencing shifted the IC50 of oxaliplatin to ±3 MAD or greater from the median IC50 of oxaliplatin in control cells, an approach (median ± 3 MAD) that has been shown to be robust to outliers and effective in controlling the false-positive rate in siRNA screens (11, 12). Second, we modeled the collective activities of the 4 individual siRNAs used for each gene by the RSA analysis (13). siRNAs with P < 0.05 values were considered significant (Supplementary Table S2). Twenty-seven high confidence hits that satisfied both of these criteria were identified (Fig. 1; Table 1) and analyzed further.

**Figure 1.** Identification of genes modulating HCT 116 tumor cell sensitivity to oxaliplatin. Results of 500-gene siRNA screen for genes that modulate sensitivity to oxaliplatin. The median log2 fold shift in the IC50 of oxaliplatin following siRNA treatment is plotted for each gene in the screen. Genes with a median IC50 shift of median IC50 ±3 MAD and greater and an RSA P < 0.05 value are indicated in red (increased resistance to oxaliplatin) or blue (increased sensitivity to oxaliplatin).
Validation of selected hits from siRNA screen

On the basis of functional categorization, we selected 12 hits from our primary screen and tested their validity with additional siRNAs. Using independently targeted pools of 4 siRNAs (ON-TARGET plus siRNAs) to reduce off-target effects and increase specificity of silencing, we categorized retested genes as validated if the resulting IC50 of oxaliplatin was shifted greater than 50% from the IC50 of oxaliplatin in cells treated with non-silencing siRNAs. All 12 of the hits selected for validation exceeded this 50% threshold (Fig. 2A).

We then examined the impact of 9 of these genes in the oxaliplatin-resistant SW480 colorectal tumor cell line (14). Silencing of each of these 9 genes (CUL4B, LTBR, MBD4, MCM3, NHEJ1, PRDX4, SFHM1, and TMEM30A), all of which conferred increased sensitivity to the HCT 116 tumor cell line, also increased sensitivity of the SW480 tumor cell line to oxaliplatin (Fig. 2B).

To independently test whether the expression of the identified genes relates to tumor cell sensitivity to oxaliplatin, we assayed the effects of overexpression of 2 genes, LTBR or TMEM30A, on tumor cell response to oxaliplatin. Transient overexpression of full-length LTBR or TMEM30A (validated by qRT-PCR; Supplementary Fig. S3B) increased the IC50 of oxaliplatin by greater than 2-fold (Fig. 2C), significantly increasing the resistance of the HCT 116 cell line to oxaliplatin, as predicted by the results with siRNA silencing.

Alterations in DNA damage and activity of signaling nodes in tumor cells with increased or decreased sensitivity to oxaliplatin

To begin to address the cellular mechanisms responsible for modulated cell sensitivity to oxaliplatin, we asked whether siRNA silencing of the identified screening hits altered the amount of DNA damage in tumor cells treated with oxaliplatin. Platinum–DNA adducts formed upon exposure to platinum-based chemotherapies are thought to be primarily removed through the nucleotide excision repair pathway (NER). Using an in vitro assay that measures the number of AP sites on the DNA of oxaliplatin-treated cells, we found that siRNA silencing of CUL4B and NHEJ1, both with known roles in the repair of DNA damage via the NER (15, 16), significantly increased the amount of DNA damage relative to oxaliplatin-treated control cells (Fig. 3A). siRNA silencing of 2 other hits with known roles in DNA replication and repair, MBD4 and MCM3 (17, 18), also increased the amount of DNA damage accumulated on treatment with oxaliplatin (Fig. 3A), although the increase did not reach statistical significance ($P < 0.05$).

Table 1. Genes conferring sensitivity or resistance to oxaliplatin

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<thead>
<tr>
<th>Symbol</th>
<th>Entrez ID</th>
<th>Full name</th>
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<td>8915</td>
<td>B-cell CLL/lymphoma 10</td>
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<tr>
<td>BCL2L10</td>
<td>10017</td>
<td>BCL2-like 10 (apoptosis facilitator)</td>
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<td>BFA1</td>
<td>51283</td>
<td>Bifunctional apoptosis regulator</td>
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<td>BRIP1</td>
<td>83990</td>
<td>BRCA1 interacting protein C-terminal helicase 1</td>
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<td>CHAF1A</td>
<td>10036</td>
<td>Chromatin assembly factor 1, subunit A (p150)</td>
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<tr>
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<td>8450</td>
<td>Cullin 4B</td>
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<td>DFFA</td>
<td>1676</td>
<td>DNA fragmentation factor, 45-kDa, α-polypeptide</td>
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<td>Interleukin 8</td>
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<td>Methyl-CpG binding domain protein 4</td>
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<td>RAD51-like 1 (S. cerevisiae)</td>
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<td>SHFM1</td>
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<td>TMEM30A</td>
<td>55754</td>
<td>Transmembrane protein 30A</td>
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<td>1026</td>
<td>Cyclin-dependent kinase inhibitor 1A (p21, Cip1)</td>
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<td>KPNA2</td>
<td>3838</td>
<td>Karyopherin α 2 (RAG cohort 1, importin α 1)</td>
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<tr>
<td>SUMO1</td>
<td>7341</td>
<td>SMT3 suppressor of mif two 3 homologue 1 (S. cerevisiae)</td>
</tr>
<tr>
<td>TP53</td>
<td>7157</td>
<td>Tumor protein p53</td>
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Silencing of the identified genes that modulate tumor cell sensitivity to oxaliplatin resulted in altered phosphorylation of several pathway signaling nodes, including mitogen-activated protein kinase cascade, JAK (Janus activated kinase)/STAT, and NF-κB pathways—pathways whose activity may be contributing significantly to changes in cell proliferation (19, 20). We carried out quantitative analyses to determine the activity of p-Akt1, p-MEK1, p-p38 MAPK, p-Stat3, and p-NF-κB p65 (Fig. 3B). Hierarchical clustering of phosphorylation levels (relative to control cells) revealed diverse and nonoverlapping clusters of pathway signaling following siRNA silencing of selected hits, with the noticeable exception of p-NF-κB p65, suggesting that multiple distinct cellular mechanisms for each hit are likely responsible for altered cell survival. Similarly, when we probed the activities of several genes regulators of apoptosis, including of p-p53, p-Bad, cleaved caspase-3, and cleaved PARP, distinct clusters of pathway activity were observed, suggesting that, upon siRNA silencing of our hits, both caspase-dependent and caspase-independent pathways regulating changes in apoptosis and/or cell death are modulated in response to DNA damage upon treatment with oxaliplatin (Fig 3C). These results again suggest that a diversity of alterations in cell signaling, including the modulation of apoptosis and necrosis, are responsible for increased tumor cell sensitivity to oxaliplatin (5).

Cell-cycle alterations in tumor cells with altered sensitivities to oxaliplatin

We also evaluated the effects that siRNA silencing of genes modulating oxaliplatin sensitivity would have on the cell cycle. Cell-cycle analysis indicates that upon treatment with oxaliplatin, all siRNA-treated cells, including those with increased siRNA-mediated resistance to oxaliplatin (CDKN1A and p53) exhibited a significant decrease in the percentage of cells in G1 with a concomitant increase in the percentage of cells in G2/M as compared with control cells (Fig 4). This is consistent with previous observations that G2/M arrest facilitates platinum-mediated cell death (21, 22), although it is interesting to note that there were no gross differences between oxaliplatin-sensitive and -resistant cells.

Network of biological processes identified as potential modulators of tumor cell sensitivity to oxaliplatin

To further understand the functional relationships between those genes whose loss of expression altered the sensitivity of tumor cells to oxaliplatin, we conducted an extensive bioinformatic analysis, using the genes identified in the initial screen (Table 1), to identify relevant networks of interacting proteins (Fig. 5). The largest interaction network is heavily populated with genes that have roles in DNA replication, recombination,
Figure 3. Functional analyses of genes modulating sensitivity to oxaliplatin. A, increased levels of DNA damage, as determined by quantification of AP sites (as % of nonsilencing siRNA-treated cells), in CUL4B- and NHEJ1-silenced HCT 116 tumor cells. Cells were transfected, treated with 1.56 μmol/L of oxaliplatin, and DNA damage was measured after 72 hours. Dashed line, 100% of control. Data represent mean ± SEM (n = 3); *, P < 0.05; NS, nonsilencing. B, hierarchical clustering of relative activities of pathway signaling nodes in cells with altered sensitivity to oxaliplatin. Heat map, the normalized log2 ratio of the phosphorylation levels of AKT1 (Ser473), MEK1 (Ser217/221), p38 MAPK (Thr180/Tyr182), STAT3 (Tyr705), and NF-κB p65 (Ser536) in test siRNA-treated cells (±1.56 μmol/L oxaliplatin), as assessed by quantitative analysis using a sandwich ELISA with epitope-specific antibodies 72 hours posttransfection and addition of oxaliplatin. C, hierarchical clustering of relative activities of key apoptotic regulators in cells with altered sensitivity to oxaliplatin. Heat map, the normalized log2 ratio of the phosphorylation levels of p53 (Ser15) and Bad (Ser112) as well as the cleavage status of PARP and caspase-3 in test siRNA-treated cells (±1.56 μmol/L oxaliplatin) relative to nonsilencing siRNA-treated cells (±1.56 μmol/L oxaliplatin), as assessed by quantitative analysis using a sandwich ELISA with epitope-specific antibodies 72 hours posttransfection and addition of oxaliplatin. Color bar, log2 of relative activity (phosphorylation or cleavage).

Conclusion

This study is an attempt to identify putative biomarkers for response to oxaliplatin, as well as potential therapeutic targets for enhancing tumor cell response to oxaliplatin. We have identified 27 genes, as well as associated cellular networks, which may serve to modulate tumor cell response to oxaliplatin.

Of the genes identified in our screen whose loss of expression results in altered tumor cell sensitivity to oxaliplatin, only 3 have been previously shown to be associated with tumor cell sensitivity to other platinum-containing chemotherapies, including cisplatin. In our study, the HCT 116 TP53+/− colon cancer cell line becomes resistant to oxaliplatin upon siRNA-mediated loss of TP53 expression (Figs. 1 and 2A), whereas previous work has shown that an HCT 116 TP53−/− clone is resistant to oxaliplatin, with a resulting G2/M arrest (25). That our data provides similar conclusions, using an orthogonal approach (e.g., siRNA knockdown), regarding the role of TP53 in increased tumor cell resistance to oxaliplatin and the resulting cell-cycle arrest, only serves to validate our experimental design and screening endpoint, as well as the biological role of TP53 in modulating response to oxaliplatin.

Bartz and colleagues, using a genome-wide siRNA screen in a TP53−/− HeLa cell line, also previously showed that the loss of SHFM1 or BRIP1 expression enhanced cisplatin cytotoxicity (26). Our study showed that the loss of either SHFM1 or BRIP1 expression increased oxaliplatin cytotoxicity in an siRNA screen by using the HCT 116 TP53+/− cell line. It is interesting to note that despite the similar mechanism of action for both cisplatin and oxaliplatin (4), only these genes (SHFM1 and BRIP1) overlapped between our study and Bartz and colleagues (Supplementary Fig. S2). Likely explanations for these differences include both the underlying mutational status of TP53 in the cell lines used and the more fundamental genomic variation between HeLa and HCT 116 tumor cell lines (derived from a human cervical adenocarcinoma and CRC, respectively), resulting in a differential sensitization upon the loss of additional genes in the presence of DNA-damaging agents such as cisplatin and oxaliplatin (26). Indeed, 11 genes that were previously identified as enhancers of cisplatin cytotoxicity...
toxicity in the study of Bartz and colleagues (26) were among the 500 genes assayed in our primary siRNA screen (Supplementary Table S1) yet were not found to alter the cytotoxicity of oxaliplatin (Supplementary Table S2). Furthermore, Bartz and colleagues showed that the loss of BRCA1 and BRCA2 selectively enhanced the cytotoxicity of cisplatin in TP53-deficient cells as compared with isogenic wild-type TP53 cells (26). These observed differences in the modulation of cytotoxicity to oxaliplatin and cisplatin underscore difficulties in identifying generalized...
predictive biomarkers for drug response, as well as the need to utilize tumors and cell lines of diverse genetic lineages for the identification of robust biomarkers for predicting drug response.

Many genes that regulate diverse aspects of DNA repair, recombination, and replication, including components of the NER, nonhomologous end-joining pathway, and mismatch repair (MMR) pathways, were represented among our hits. The NER portion of this module includes CUL4B, an E3 ubiquitin ligase that binds to damaged DNA and ubiquitinates histone H2A, modifying the structure of chromatin and facilitating NER (15, 27). NHEJ1 encodes a DNA repair protein that is essential for the nonhomologous end-joining pathway, which preferentially mediates repair of double-stranded breaks in DNA (16, 28).

The defects in the MMR has long been appreciated as a possible mechanism of resistance to chemotherapy by directly impairing the ability of the tumor cell to detect DNA damage, faithfully replicate DNA, and ultimately the activation of apoptosis (29). Our data identify the MMR pathway genes MSH4, MBD2, and MBD4 as drivers of oxaliplatin resistance (30).

Alterations in DNA replication due to defects in the recruitment of minichromosome maintenance (MCM) proteins onto chromatin during the assembly of the pre-replication complex at origins of replication (18, 31, 32), while not previously implicated in drug resistance, may cause tumor cells to develop resistance to chemotherapies. Indeed, MCM3, MCM4, and MCM6, all components of the MCM complex, were identified as modulators of tumor cell sensitivity to oxaliplatin.

The mechanisms through which tumor cells may acquire altered sensitivity to oxaliplatin have been thought to be linked to the DNA-damaging activity of oxaliplatin (e.g., NER, MMR; ref. 4). However, in addition to DNA replication, several gene hits from our siRNA screen have functional annotations not previously implicated in the modulation of tumor cell sensitivity to oxaliplatin—several of which may have heretofore unappreciated roles in DNA damage response. LTBR, lymphotoxin-β receptor, is a member of the TNF receptor (TNFR) superfamily that stimulates the noncanonical NF-κB signaling and cell death via the recruitment of TNFR-associated factor 3 (TRAF3) to the LTBR cytoplasmic domain (19, 33) and plays an important role in the pathobiology of hepatocellular carcinoma (34). PRDX4, an additional hit from our screen, is thought to activate NF-κB by modulating IκB-α phosphorylation (35).

It is noteworthy that siRNA knockdown of the gene ERCC1 did not alter tumor cell sensitivity to oxaliplatin. Multiple prior studies have shown that in vitro and in vivo, mRNA levels of ERCC1 are modest predictors of tumor cell sensitivity and patient response to cisplatin (36–39). However, the utility of ERCC1 in predicting patient response...
to oxaliplatin is limited to combination 5-FU/oxaliplatin chemotherapies in the clinical setting (40, 41). Our data, while not specifically identifying ERCC1 as a candidate hit, did identify numerous genes involved in the repair of DNA damage, including members of the NER (of which ERCC1 is a key molecule) and DNA MMR pathways (Table 1). It is possible that ERCC1 mRNA levels may predict resistance to oxaliplatin only in the clinical setting of combination therapy with 5-FU (42). In our screen, we treated cells with oxaliplatin alone to reduce the identification of false-positive hits and to simplify the screen design. It is also possible that our assay endpoint, by design, resulted in a particularly stringent cutoff for hit selection that yielded only very robust modulators of tumor cell sensitivity to oxaliplatin.

Our results indicate that diverse, and possibly, redundant processes may influence cell proliferation and survival upon treatment with oxaliplatin. That multiple processes may function redundantly to drive cell resistance to DNA-damaging agents might make it difficult to find robust biomarkers to predict patient response to oxaliplatin. Finally, protein products of the identified genes suggest therapeutic strategies to sensitize tumor cells to oxaliplatin and other DNA-damaging chemotherapeutic agents.

Disclosure of Potential Conflicts of Interest

K. Harradine, J. Baker, and R. Pelham are employees of Genomic Health, Inc.

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