Role of Insulin-Like Growth Factor-1 Signaling Pathway in Cisplatin-Resistant Lung Cancer Cells

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Received Jan 18, 2011, and in revised form Jun 3, 2011. Accepted for publication Jun 24, 2011

Summary

This study identified molecular differences between cisplatin-resistant and parental H460 lung cancer cells by using microarray expression analysis. Cisplatin-resistant cells illustrated more rapid in vivo tumor growth and greater survival following treatment with cisplatin or radiation than parental H460 cells. Cisplatin-resistant cells also demonstrated decreased expression of insulin-like growth factor binding protein-3 (IGFBP-3) and increased IGF-1R signaling. Cisplatin resistance was reversed by treating cisplatin-resistant cells with human recombinant IGFBP-3 and siRNA targeting IGF-1 receptor.

Purpose: The development of drug-resistant phenotypes has been a major obstacle to cisplatin use in non—small-cell lung cancer. We aimed to identify some of the molecular mechanisms that underlie cisplatin resistance using microarray expression analysis.

Methods and Materials: H460 cells were treated with cisplatin. The differences between cisplatin-resistant lung cancer cells and parental H460 cells were studied using Western blot, MTS, and clonogenic assays, in vivo tumor implantation, and microarray analysis. The cisplatin-R cells were treated with human recombinant insulin-like growth factor (IGF) binding protein-3 and siRNA targeting IGF-1 receptor.

Results: Cisplatin-R cells illustrated greater expression of the markers CD133 and aldehyde dehydrogenase, more rapid in vivo tumor growth, more resistance to cisplatin- and etoposide-induced apoptosis, and greater survival after treatment with cisplatin or radiation than the parental H460 cells. Also, cisplatin-R demonstrated decreased expression of insulin-like growth factor binding protein-3 and increased activation of IGF-1 receptor signaling compared with parental H460 cells in the presence of IGF-1. Human recombinant IGF binding protein-3 reversed cisplatin resistance in cisplatin-R cells and targeting of IGF-1 receptor using siRNA resulted in sensitization of cisplatin-R-cells to cisplatin and radiation.

Conclusions: The IGF-1 signaling pathway contributes to cisplatin-R to cisplatin and radiation. Thus, this pathway represents a potential target for improved lung cancer response to treatment.

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Keywords: Cisplatin resistance, Insulin-like growth factor binding protein-3, IGFBP-3, Lung cancer, Radiotherapy
introduction

Lung cancer is the leading cause of cancer-related deaths worldwide (1). Cisplatin-based combination treatments have been the conventional management for advanced non–small-cell lung cancer (NSCLC) for more than two decades; however, a major obstacle in using this drug has been the development of cisplatin resistance (2). Therefore, the development of more effective treatment targeting molecules associated with resistance is necessary to improve outcomes.

Cisplatin is frequently used with radiotherapy in the treatment of NSCLC. Patients who received cisplatin-based treatment followed by radiotherapy have been noted to have a correlation between their response to cisplatin and the subsequent response to radiotherapy (3). In addition, in vitro studies have revealed that the acquisition of cisplatin resistance in cell lines can result in the acquisition of cross resistance to radiotherapy (4). Thus, identifying the molecular mechanisms associated with cisplatin resistance might provide a target to overcome resistance to combined modality treatment.

High-throughput techniques comparing the gene signature of cisplatin-resistant cells with normal cancer cells have revealed genes that are differentially expressed between these two cell populations. In the present study, cells isolated after cisplatin exposure (cisplatin-R) expressed markers associated with lung cancer stem cells. Microarray gene expression analysis comparing cisplatin-R cells with parental H460 cells found that insulin-like growth factor-binding protein-3 (IGFBP-3) was a highly ranked hub gene that was downregulated in cisplatin-R cells.

Insulin-like growth factor binding protein-3 regulates IGF-1 bioactivity by sequestering IGF-1 in the extracellular milieu, thereby inhibiting its mitogenic and antipapoptotic actions (5). Overexpression of IGFBP-3 inhibits the growth of NSCLC cells by inducing apoptosis (6). Reduced IGFBP-3 expression in NSCLC has been associated with decreased tumor cell sensitivity to cisplatin (7). Therefore, we investigated the role of IGFBP-3 and the insulin-like growth factor-1 resistance (IGF-1R) pathway in chemotherapy- and radiation-resistant cells and its potential as a treatment target in NSCLC. We found that IGF-1R is highly active in cisplatin-R cells and that small interfering RNA (siRNA) treatment of cisplatin-R cells results in the recovery of their sensitivity to cisplatin and radiotherapy. Thus, the IGF-1/IGF-1R pathway holds promise as a therapeutic target to overcome resistance to chemotherapy and radiotherapy in NSCLC.

Methods and Materials

Cell lines and reagents

National Cancer Institute (NCI)-H460 cells were obtained from the American Type Culture Collection. The cells were grown in Roswell Park Memorial Institute 1640 culture medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA).

Cisplatin-R cells were selected as previously described (8). In brief, after the H460 cells were treated with 3 μM cisplatin for 7 days, the survival cells were trypsinized and cultured in 0.8% methyl cellulose that was supplemented with 20 ng/mL epidermal growth factor (BD Biosciences Bedford, MA), basic fibroblast growth factor, and 4 μg/mL insulin (Sigma Aldrich, St. Louis, MO). Epidermal growth factor, basic fibroblast growth factor (20 ng/mL), and insulin (4 μg/mL) were added every second day for 14 days to allow the cells to form spheres. The spheres were diluted with phosphate-buffered saline (PBS) to make a single-cell suspension and then plated in 100-mm dishes with Roswell Park Memorial Institute 1640 supplemented with 10% fetal bovine serum. Cisplatin and etoposide were obtained from Sigma Aldrich. Human recombinant IGF-1 and human recombinant IGFBP-3 (hrIGFBP-3) were purchased from R&D Systems (Minneapolis, MN). 5’AZA-2’DC was obtained from Sigma Aldrich, and the cells were treated with 10 μM for 72 h.

RNA extraction and microarray

The cells were plated in 6-well plates and allowed to reach 80% confluency. Next, 1 mL of Trizol (Invitrogen, Carlshbad, CA) was added into each well, and RNA was then extracted according to the manufacturer’s guidelines. The RNA was further purified using the RNAeasy kit (Qiagen Germantown, MD). Sample integrity was confirmed using the Agilent Bioanalyzer (Agilent, Santa Clara, CA), and then the samples were quantitated at 260 nm using the Nanodrop spectrophotometer (ThermoFisher Scientific, Wilmington, DE). Next, 200 ng of the total input RNA was used in Affymetrix Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA) for the target labeling reactions. The reactions, hybridization, and data processing were performed in the Vanderbilt Functional Genomics Shared Resources according to the manufacturer’s protocol using the Affymetrix reagent kits (catalog no. 900652). Three biologic replicates were profiled for each cell line. The microarray data were normalized using the robust multichip average method (9), and differential genes were identified using both the significance analysis of microarrays (false discovery rate <0.1) and a fold change >2. The microarray data was submitted to Gene Expression Omnibus (Gene Expression Omnibus ID no. GSE21656). Additional details are provided in the “Supplementary Methods” section.

siRNA and transfections

Parental and cisplatin-R H460 cells were transfected 24 h after seeding in a six-well plate. IGF-1R siRNA and control siRNA (Santa Cruz Biotechnology, Santa Cruz, CA; 25 pmol) in 100 μL of serum-free, antibiotic-free, opt-MEM (Invitrogen) were mixed with 5 μL Lipotectamine RNAimax transfection reagent (Invitrogen) and dissolved in 100 μL of the same medium and allowed to stand at room temperature for 20 m. The 200 μL transfection solutions were added to each well containing 2 mL medium and incubated for 6 h before being replaced with 2 mL fresh medium supplemented with 10% fetal bovine serum and antibiotics.
Cell viability assay

MTS assay was performed using tetrazolium compound-based CellTititer 96 AQueous One Solution Cell Proliferation assay (Promega Madison, WI). Parental and cisplatin-R cells were seeded in 96-well plates at 2,000 cells/well. The cells were treated with varying concentrations of cisplatin the next day. For the siRNA studies, the cells were transfected 48 h before treatment with cisplatin. For hrIGFBP-3 treatment, the cisplatin-R cells were seeded at 5,000 cells/well and allowed to attach overnight before exposure to 30 μg/mL hrIGFBP-3 or 3 μM cisplatin in serum-free Roswell Park Memorial Institute1640, either alone or in combination. MTS assay was performed 72 h after treatment.

Western blot analysis

The cells were washed twice with ice-cold PBS and then lysed in M-Per mammalian lysis buffer (ThermoFisher Scientific). The protein concentration of the lysates was determined with the Bradford reagent (BioRad Hercules, CA), and equal amounts of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis of a 10% or 15% gel. The separated proteins were transferred to a nitrocellulose membrane, which was then exposed to 5% nonfat dried milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature and incubated overnight with antibodies against aldehyde dehydrogenase (ALDH) (R&D Systems Minneapolis, MN), CD133 (Abcam), caspase-3, phospho-IGF-IR (Tyr1135/1136), total IGF-IR (Cell Signaling Technology), IGFBP-3 (R&D Systems), or actin (Sigma Aldrich). The membranes were then washed with Tris-buffered saline containing 0.1% Tween 20 before being incubated with horseradish peroxidase-conjugated goat antibodies to rabbit or mouse (Santa Cruz Biotechnology). Immune complexes were detected with chemiluminescence reagents (Perkin-Elmer Life Science Waltham, Massachusetts).

Reverse transcriptase-polymerase chain reaction

A total of 2 μg of total RNA was reverse transcribed using hexamer primer and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) in a final volume of 20 μL. Then, 2 μL of the cDNA was used for the polymerase chain reaction (PCR) to amplify the IGFBP-3 gene. The sequences of the primers used have been previously described (7). Actin was used as a control.

DNA extraction, bisulfite treatment, and MSP assay

Genomic DNA was isolated using DNeasy kit (Qiagen) from parental and cisplatin-R H460 cells. For bisulfite treatment, the extracted DNA (1 μg) was modified using the EZ DNA Methylation Kit (Zymo Research, Orange, CA). The methylation-specific primers were designed according to the CpG island sequence from a gene browser (available from: http://genome.ucsc.edu/). The MSP primers are designed using methprimer (available from: http://www.urogene.org/methprimer/index1.html). The primers used to amplify the methylated (M) IGFBP-3 promoter were M(S) 5′-AATCGTAGAAGATATTAAAATTCGA-3′ and M (AS) 5′-AAC CAAAAAAAATAAACACGTF-3′. The primers for the unmethylated (U) IGFBP-3 were U(S) 5′-AATTGTAGAGAT ATTAAAATTGTA-3′ and U(AS) 5′-ACCAAAAAAATATAACAAACACATT-3′. Bisulfite-modified DNA was mixed with 10× PCR buffer, 150 μM of deoxynucleotide triphosphate, 0.3 μM of primer, and 1 U of HotStarTaq (Qiagen). The PCR condition for both methylated and unmethylated promoter consisted of 15 min at 95°C, 40 cycles for 15 s at 94°C, 30 s at 55°C, and 30 s at 72°C followed by 10 min of elongation. The PCR products were resolved by electrophoresis in 3% agarose gels containing ethidium bromide.

Clonogenic survival assay

The cells were irradiated with 0–6 Gy (dose rate, 1.8 Gy/min) using a 137Cs irradiator (J.L. Shepherd and Associates, Glasgow, Scotland). After irradiation, the cells were incubated at 37°C for 8–10 days. The cells were fixed for 15 min with 3:1 methanol/ acetic acid and stained for 15 min with 0.5% crystal violet (Sigma Aldrich) in methanol. After staining, the colonies were counted by the naked eye (cutoff of 50 viable cells). The surviving fraction was calculated as follows: (mean colony counts)/(cells inoculated) × (plating efficiency), with plating efficiency defined as (mean colony counts)/(cells inoculated for irradiated controls). The dose enhancement ratio was calculated as the dose of radiation that yielded a surviving fraction of 0.2 for control siRNA-treated cells divided by that for IGF-IR siRNA-treated cells.

Animals and tumor xenograft assay

All animal studies were approved and handled according to the Institutional Animal Care and Use Committee guidelines (approved protocol M/08/095). Female athymic nude mice (5–6 weeks old) were purchased from Harlan Laboratories (Indianapolis, IN). Exponentially growing parental H460 and cisplatin-R H460 cells were trypsinized and washed with PBS and then diluted into 1 × 106 cells/100 μL PBS. The cell suspension was injected subcutaneously into the left or right flank of each mouse (n = 5). After tumor visibility, the tumor size was measured with a digital caliper every 2 days. The tumor volume was calculated as the (length × width × height)/2.

Statistical analysis

All data are presented as the mean ± standard deviation. A two-tailed Student’s t test was performed to determine the statistically significant differences between the two groups. The Wilcoxon two-sample test was performed to compare the times required for the tumor size to quadruple in a xenograft model.

Results

Cisplatin-R H460 cells exhibit greater levels of markers associated with lung cancer stem cells and demonstrate more aggressive tumor growth

Lung cancer stem cells are known to express specific cell surface markers, including CD133 (10) and ALDH (11). The cell lysates of parental and cisplatin-R H460 cells were probed for CD133 and ALDH and subjected to Western blot analysis. Greater levels of CD133 and ALDH were detected in the cisplatin-R cells than in the parental cells (Fig. 1A). An equal number of parental or cisplatin-R H460 cells (1 × 106) were inoculated into nude mice, and the
quantitation of tumor growth demonstrated faster growth in the cisplatin-R xenografts (Fig. 1B). The tumor volume quadrupling time was significantly shorter in cisplatin-R xenografts (5.4 days; 95% confidence interval, 4.9–5.9) compared with parental H460 xenografts (7.8 days; 95% confidence interval, 7.4–8.2; \( p < .03 \)).

Cisplatin-R H460 cells are resistant to treatment with radiation and cisplatin

To determine the differences in the response of parental and cisplatin-R cells to chemotherapy, the cells were treated with either cisplatin (20 \( \mu \text{M} \)) or etoposide (100 \( \mu \text{M} \)) for 16 h. Cleavage of caspase-3 was detected using Western blotting as a marker for chemotherapy-induced apoptosis. Significant attenuation of apoptosis was observed in cisplatin-R cells compared with the parental controls (Fig. 2A). To determine whether cisplatin-R cells were also resistant to radiation-induced apoptosis, both cell types were irradiated with 20 Gy and collected 48 h later. Immunoblotting demonstrated less caspase-3 cleavage in cisplatin-R H460 cells than in parental cells (Fig. 2B). These data suggest that cisplatin-R H460 cells are more resistant to the apoptosis induced by either chemotherapy or radiotherapy. Consistent with less apoptosis seen by Western blotting, the MTS

![Image](image1)

Fig. 1. Cisplatin-resistant (CDDP-R) H460 cells exhibited greater levels of markers associated with lung cancer stem cells and demonstrate more aggressive tumor growth. (A) Aldehyde dehydrogenase (ALDH) and CD133 detected by immunoblotting of total cell lysates of parental and cisplatin-resistant cells. (B) Growth curves showing parental and cisplatin-resistant mean tumor volumes and standard errors (\( n = 6 \)). \( *p < .05; \) \( **p < .001 \).

![Image](image2)

Fig. 2. Cisplatin-resistant (CDDP-R) H460 cells resistant to chemotherapy and radiotherapy (RT). (A, B) Parental and cisplatin-resistant H460 cells exposed to cisplatin (20 \( \mu \text{M} \) for 16 h), etoposide (100 \( \mu \text{M} \) for 16 h), or 20 Gy radiation, and cleaved caspase-3 was detected by Western blotting as a marker for chemotherapy-induced apoptosis. Significant attenuation of apoptosis was observed in cisplatin-R cells compared with the parental controls (Fig. 2A). To determine whether cisplatin-R cells were also resistant to radiation-induced apoptosis, both cell types were irradiated with 20 Gy and collected 48 h later. Immunoblotting demonstrated less caspase-3 cleavage in cisplatin-R H460 cells than in parental cells (Fig. 2B). These data suggest that cisplatin-R H460 cells are more resistant to the apoptosis induced by either chemotherapy or radiotherapy. Consistent with less apoptosis seen by Western blotting, the MTS
cell viability assays showed ~40% of cisplatin-R cells survived after 20 μM of cisplatin treatment compared with 15% of parental cells (Fig. 2C). The clonogenic assay results confirmed these findings (data not shown). We observed a plating efficiency of 49% in cisplatin-R cells (dimethyl sulfoxide, 117%) after 1 μM cisplatin compared with a plating efficiency of 17% in the parental cells (dimethyl sulfoxide, 103%). Similarly, the clonogenic assay showed that cisplatin-R cells were more resistant to radiation (dose enhancement ratio, 1.21; p < .01) than parental cells (Fig. 2D).

**Table**  
Top 10 genes based on joint rank score

<table>
<thead>
<tr>
<th>Gene ID</th>
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<th>Gene name</th>
<th>JRS</th>
<th>Fold change</th>
<th>Degree</th>
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<tr>
<td>3486</td>
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<td>Contactin 1</td>
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<td>5.41</td>
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<tr>
<td>4739</td>
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<tr>
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</table>

Abbreviation: JRS = joint rank score.

**Fig. 3.** Holistic view of protein subnetwork shows insulin-like growth factor-binding protein-3 and its interacting proteins. Subnetwork with insulin-like growth factor-binding protein-3 and the full perturbed subnetwork (main view and inset, respectively). Insulin-like growth factor-binding protein-3 node boxed in both views. Each node represents a gene and connecting lines represent physical protein interactions between end nodes. Color changes reflect expression fold changes of cisplatin-resistant cells compared with parental cells for each gene (red, increased expression; green, decreased expression).
Reduced IGFBP-3 level in cisplatin-R H460 cells

To determine the potential molecular differences that contributed to the chemotherapy resistance and radioresistance of cisplatin-R cells, microarray analyses were performed to compare the expression profiles of the parental and cisplatin-R cells. Of the 20,364 genes examined in the microarray, 180 were found to be differentially expressed genes. Of these genes, 73 were upregulated in cisplatin-R cells and 107 were downregulated. A heatmap is provided in the “Supplementary Methods” section. Next, a protein interaction network was integrated with the microarray data. The protein interaction network consists of 10,374 nodes and 50,909 edges. The nodes represent gene products, and the edges represent physical interactions between the interconnected nodes. Differentially expressed genes were used as seeds for a perturbed subnet-work (PSN) search. This analysis resulted in a PSN with 212 genes and 404 edges. The giant component size, a measure of the group of nodes with the greatest number of members, was 195 (92%), indicating that most of the PSN is connected. We then used a data fusion model to prioritize the genes considering both their degrees in the PSN and the change in expression. A joint rank score was calculated ranking highest according to the fold change (4.3) and the degree (12) in the protein interaction network; thus, it was selected as a prioritized gene for additional characterization (Table and Fig. 3). Additional details regarding the bioinformatics analyses are provided in the supplementary methods.

DNA hypermethylation plays a role in the inactivation of genes and subsequent treatment-resistant phenotypes in cancer. To determine whether hypermethylation contributes to the reduced levels of IGFBP-3 seen in cisplatin-R cells, the cells were treated with the de-methylator 5′AZA-2′DC. Western blotting and reverse transcriptase-PCR were performed on the cells to determine how de-methylation affected the IGFBP-3 protein and DNA levels. The treatment with 5′AZA-2′DC increased the IGFBP-3 protein and DNA levels in both parental and cisplatin-R cells, suggesting that methylation is at least partially responsible for downregulating IGFBP-3 levels (Fig. 4A). The MSP assay results confirmed increased IGFBP-3 promoter methylation in the cisplatin-R cells compared with the parental H460 cells (Fig. 4B).

IGF-1 signaling is enhanced in cisplatin-R H460 cells and addition of hrIGFBP-3 reverses cisplatin resistance in cisplatin-R cells

To determine whether the reduced levels of the IGFBP-3 transcript resulted in lower levels of IGFBP-3 protein, Western blot analysis was performed. Decreased levels of IGFBP-3 were expressed in cisplatin-R cells than in parental H460 cells (Fig. 5A). Because IGFBP-3 sequesters IGF-1 in the extracellular space, decreased levels of IGFBP-3 likely result in increased downstream activation of the IGF-1R. To test this hypothesis, immunoblots probing phosphorylated IGF-1R were performed after induction by recombinant IGF-1. A greater induction of phospho-IGF-1R was observed in cisplatin-R H460 cells than in the parental cell line (Fig. 5A, B) suggesting that cisplatin-R cells can upregulate the IGF1-R signaling pathway to a greater extent than parental H460 cells. To confirm that decreased IGFBP-3 expression contributes to the ability of cisplatin-R cells to upregulate IGF-1R signaling in response to IGF-1, hrIGFBP-3 was added to cells in increasing concentrations, which reversed this effect (Fig. 5B).

Cisplatin-R cells were treated with hrIGFBP-3 that sequesters IGF-1 and prevents binding to the IGF-1R. As shown by the MTS assay results, the cisplatin-R cells treated with 30 μg/mL hrIGFBP-3 before 3 μM cisplatin were significantly more sensitive to treatment than cisplatin-R cells treated with cisplatin alone (Fig. 5C).

IGF-1R inhibition results in sensitization to radiation and cisplatin

To determine whether inhibition of the IGF-1R signaling pathway could sensitize cisplatin-R cells to cisplatin and radiation, siRNA against IGF-1R was transfected into the parental and cisplatin-R cell lines. Western blotting showed that the IGF-1R level was dramatically reduced at 48 h after transfection (Fig. 6A). The MTS assay results showed that inhibition of IGF-1R signaling by siRNA enhanced cisplatin-induced cell death in cisplatin-R cells and in parental cells (Fig. 6B). Similarly, the clonogenic survival assays showed that siRNA against IGF-1R sensitized cisplatin-R cells to radiation (dose enhancement ratio, 1.17; \( p < .05 \)). However, this time had no effects on the parental cells (Fig. 6C).
Taken together, these findings suggest that the IGF-1 pathway is a potential target to overcome cisplatin resistance and radiation resistance in lung cancer.

**Discussion**

In the present study, we described cisplatin-R cells that expressed greater levels of the cell surface markers CD133 and ALDH (Fig. 1A) compared with parental H460 cells. These markers have been found in lung cancer stem cells and have been associated with cells that have stem cell-like properties (10–12). Although CD133 expression has been correlated with treatment resistance, studies have not shown it to be a prognostic marker for the survival of NSCLC patients (8). In addition, CD133-positive and CD133-negative A549 and H446 lung cancer cell subpopulations contain similar numbers of cancer stem cells, suggesting that the precise role of CD133 in cancer stem cells is still undiscovered (13). However, high ALDH1 protein expression has been identified as a poor prognostic marker, possibly because ALDH-positive lung tumor cells have more lung cancer stem cells than ALDH-negative cells (14). ALDH-positive cells isolated from NCI-H358 and NCI-H125 lung cancer cell lines showed enrichment of tumorigenic CD133-positive cancer cells (15). Because the precise role of these cell surface markers has not been completely deciphered, the expression of CD133 and ALDH is not sufficient to suggest that cisplatin-R cells are cancer stem cells. However, these findings hint that additional testing of stem cell properties in cisplatin-R cells is warranted.

Cisplatin-R cells in a xenograft lung cancer model quadrupled the tumor size more rapidly (Fig. 1B) than did the parental H460 cells. This suggests that resistant cells might be more aggressive than their parental cells. In addition, cisplatin-R cells were also more resistant to both chemotherapy and radiation (Fig. 2) compared with the parental H460 cells. These data are consistent with previous findings that suggest that cells that acquire cisplatin resistance have an increased potential for tumor formation.
resistance develop cross resistance to radiotherapy through similar mechanisms (4, 16).

To elucidate genes that play a role in the differential phenotype of cisplatin-R cells, microarray analyses were performed (Fig. 3). The gene expression data were ranked as described in the “Results” section (Table). The most highly ranked gene was IGFBP-3, which modulates the IGF-1R signaling pathway. Thus, it is possible that this pathway contributes to the acquisition of treatment resistance by cisplatin-R cells. Remarkably, the second-most highly ranked gene encodes transglutaminase, which was recently shown to protect epithelial ovarian cancer cells from cisplatin-induced apoptosis, providing an internal control for our microarray data collecting and processing (17).

Our finding that IGFBP-3 is suppressed in cisplatin-R cells is consistent with previous studies. Hypermethylation of the IGFBP-3 promoter might contribute to this suppression (7). Such hypermethylation is seen in 61.5% of Stage I NSCLC patients and is associated with a poor prognosis (18), in addition to contributing to cisplatin resistance in NSCLC cells (7). We found that treatment of the cisplatin-R cells with the demethylator 5'-AZA-2'-DC increased both DNA and protein levels of IGFBP-3 (Fig. 4), further suggesting hypermethylation as a mechanism of suppression. Furthermore, treatment of cisplatin-R cells with hrIGFBP-3 recovered sensitivity to cisplatin (Fig. 5C). This suggests that the suppressed IGFBP-3 levels observed in cisplatin-R cells partially contribute to the cisplatin-resistant phenotype of these cells. Similarly, studies of lung and colon cancer cells have shown tumor inhibition with hrIGFBP3 (19). On the basis of preliminary data, research assessing the feasibility or effectiveness of IGFBP3 analogs for cancer treatment in humans is warranted.

In concordance with decreased IGFBP-3, cisplatin-R cells showed increased IGF-1R signaling in the presence of IGF-1 compared with that in the parental H460 cells (Fig. 6). We hypothesized that these differences might contribute to the treatment resistance of cisplatin-R cells (Fig. 7). Increased IGF-1R activation in breast cancer cell lines leads to increased Akt activation and resistance to tamoxifen (20). Inhibition of IGF-1R signaling has previously been found to increase the sensitivity of NSCLC cells to radiation (21) and to enhance the sensitivity of small-cell lung cancer to etoposide and carboplatin (22). Using the MTS and clonogenic assays, we have demonstrated that the knockdown of IGF-1R rescued the cisplatin and radiation sensitivity of cisplatin-R cells. By employing microarray analysis and gene expression studies, we have identified potential targets for overcoming drug resistance in cisplatin-R cells.
sensitivity of cisplatin-R cells (Fig. 6). These findings suggest that this pathway represents a promising target for overcoming treatment resistance. However, >30 agents that target IGF-1R are already in preclinical or clinical studies and seem to have a favorable toxicity profile (23). In a recent Phase II study, NSCLC patients had greater responses rates when treated with the IGF-1R inhibitor, figitumumab, plus paclitaxel and carboplatin than when treated with paclitaxel and carboplatin alone (54% vs. 42%, respectively) (24).

Profiling the gene expression of cisplatin-R cells is important for identifying potential targets for overcoming therapeutic resistance. The molecular profiling of tumor tissue from NSCLC patients allows tailoring of individualized therapy. The gene expression profiling for cisplatin resistance in lung cancer could be an important first step in heralding the age of personalized medicine.

References


Fig. 7. Cisplatin-resistant cells have greater insulin-like growth factor (IGF) pathway activation than parental H460 cell line. Binding of IGF binding protein-3 to IGF-1 prevents receptor binding and downstream pathway activation. With decreased expression of IGF binding protein-3 in cisplatin-resistant cells, IGF-1 signaling through IGF-1 receptor is maximized, leading to increased metabolism, proliferation, and survival through Ras-raf-mitogen activated protein kinase (MAPK) and PI3K-PDK/ATK-TOR-S6K pathways.


