Genetic variation that predicts platinum sensitivity reveals the role of miR-193b* in chemotherapeutic susceptibility

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Running Head: miR-193b* affects platinum sensitivity

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Abstract

Platinum agents are the backbone of cancer chemotherapy. Recently we identified and replicated the role of a single nucleotide polymorphism (SNP, rs1649942) in predicting platinum sensitivity both in vitro and in vivo. Using the CEU samples from the International HapMap Project, we found the same SNP to be a master regulator of multiple gene expression phenotypes, prompting us to investigate whether rs1649942-mediated regulation of microRNAs (miRNAs) may in part contribute to variation in platinum sensitivity. To these ends, sixty unrelated HapMap CEU I/II samples were utilized for our discovery-phase study using high-throughput genome-wide miRNA and gene expression profiling. Examining the relationships among rs1649942, its gene expression targets, genome-wide miRNA expression and cellular sensitivity to carboplatin and cisplatin, we identified 2 platinum-associated miRNAs (miR-193b* and miR-320) that inhibit the expression of five platinum-associated genes (CRIM1, IFIT2, OAS1, KCNMA1 and GRAMD1B). We further replicated the relationship between the expression of miR-193b*, CRIM1, IFIT2, KCNMA1 and GRAMD1B, and platinum sensitivity in a separate HapMap CEU III dataset. We then showed that over-expression of miR-193b* in a randomly selected HapMap cell line results in resistance to both carboplatin and cisplatin. This relationship was also found in 7 ovarian cancer cell lines from NCI60 dataset and confirmed in an ovarian cancer cell line (OVCAR-3) that over-expression of miR-193b* leads to increased resistance to carboplatin. Our findings highlight a potential mechanism of action for a previously observed genotype-survival outcome association. Further examination of miR-193b* in platinum sensitivity in ovarian cancer is warranted.
**Abbreviation:** CEU, Utah residents with northern and western European ancestry; eQTL, expression quantitative trait locus; LCLs, lymphoblastoid cell lines; miRNA, microRNA; SNPs, single nucleotide polymorphisms
Introduction

Platinum agents, including carboplatin and cisplatin, are the backbone of classic chemotherapeutic regimens. They are used in the treatment of a wide array of cancers (1); however inter-individual variation in response to these agents is observed frequently without a clearly understood mechanism of resistance. Recently, utilizing a genome-wide cell-based approach, we have identified a set of germline genetic variations in the form of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) that predict platinum sensitivity (2-4). Importantly, several of these cell-based findings have been validated in clinical patient samples (5, 6).

In particular, a SNP (rs1649942) was found to be associated with both carboplatin and cisplatin sensitivity in vitro (3, 5). Notably, this SNP was, in addition, found to be associated with progression free survival (PFS) and overall survival (OS) in 377 non-hispanic white ovarian cancer patients who had undergone carboplatin and taxol treatment (5), although the association was not supported in a (putatively more) heterogeneous set of patients (5), and with decrease in platelet counts in head and neck cancer patients who were treated with platinum-based induction therapy (6). Our group has shown that rs1649942 is a master regulator of multiple gene expression traits in a comprehensive genome-wide expression quantitative trait loci (eQTL) study (7). Many of these expression targets of rs1649942 are also correlated with platinum sensitivity. Interestingly, all observed SNP-gene expression relationships are trans- relationships, suggesting a possible indirect SNP effect on gene expression mediated by other (intermediate) transcriptional regulators.
microRNAs (miRNAs), a class of non-coding small RNA molecules that play an important role in the post-transcriptional regulation of gene expression, are predicted to target a third of all human mRNAs (8, 9). Recent studies have demonstrated the role of miRNAs in diverse cellular, developmental and pathological processes (10). Specifically, it was reported that miRNAs are potential mechanisms of resistance to cytotoxic anticancer therapy (11). Therefore, in this study, we sought to evaluate the role of miRNAs in mediating the effect of rs1649942 on platinum sensitivity. The identification of such miRNAs promises to not only improve our understanding of the role of this important class of non-coding RNAs in mediating pharmacologic traits, but also to provide potential biomarkers or druggable targets for platinum resistance.
Materials and Methods

Cell lines and drugs. Unrelated International HapMap lymphoblastoid cell lines (LCLs; 60 CEU (Utah residents with northern and western European ancestry; HAPMAPPT01) were purchased from Coriell Institute for Medical Research (Camden, NJ) for genome-wide discovery; we refer to these cell lines as “discovery-phase samples”. Fifty eight unrelated HapMap CEU III (HAPMAPPT06) LCLs were obtained from the same resource and used for replication experiments; we refer to these LCLs as “replication samples”. One benefit of the use of these cell lines for pharmacogenomic studies is that extensive genome-wide genotype and gene expression data are publicly available, which enables us to conduct genotyping assays on randomly chosen cell lines using randomly selected SNPs to authenticate the cell lines. This genotyping assay is regularly performed in our Pharmacogenomic of Anticancer Agent Research (PAAR) cell line and genotyping core; furthermore, the cell lines have undergone extensive genetic studies by the International HapMap Consortium (12) and the 1000 Genomes Project (13). LCLs were maintained in RPMI 1640/1% l-glutamine plus 15% FBS as previously described (14). Ovarian cancer cell line SKOV-3 was purchased from ATCC. Carboplatin, cisplatin, and DMSO were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

mRNA and miRNA expression quantification. In the discovery-phase samples, global baseline gene expression on 87 CEU LCLs were quantified using the Affymetrix GeneChip® Human Exon 1.0 ST array (Affymetrix exon array) as previously described (15) (GEO accession No: GSE7761). Baseline miRNA expression was evaluated in 53 CEU HapMap LCLs using Exiqon miRCURY™ LNA arrays v10.0 (Exiqon array). After
normalization and filtering, 201 miRNAs were found to be expressed in these samples (16).

In the replication samples, quantitative PCR (qPCR) methods were utilized to measure the expression of 5 genes (CRIM1, IFIT2, OAS1, KCNMA1 and GRAMD1B) and 2 miRNAs (miR-193b* and miR-320a). All miRNA primers were purchased from Exiqon while Applied Biosystems Taqman primer/probe sets were used to quantify mRNA expression. Real-time PCR was conducted using an ABI Vii7 thermocycler (Applied Biosystems, Foster City, CA). Details for total RNA isolation, cDNA conversion and PCR condition were described in our previous publication (17).

**Cellular sensitivity to platinum agents.** Both discovery and replication LCLs were treated with increasing dosage of either carboplatin or cisplatin for 72 and 48 hours, respectively. Cellular growth inhibition was measured using an Alamar Blue assay as described previously (14). Drug concentration required to inhibit 50% of cellular growth is defined as IC$_{50}$ and was used to characterize individual cellular sensitivity to platinum agents. The cellular sensitivity to carboplatin and cisplatin in various HapMap LCLs including those studied here was reported elsewhere (14, 18) and all data as well as genome-wide SNP association results with these data have been made publicly available (19).

**Data analysis.** The overall workflow in the discovery phase is illustrated in Fig 1. In the discovery-phase samples, linear regression was performed between the rs1649942 genotype, assuming an additive model, and the 201 miRNA expression levels obtained through Exiqon arrays. Only those miRNAs that were associated with rs1649942 at p<0.05 were further examined for (negative) correlation with the expression of 39 genes.
that were expression targets of the SNP as an eQTL (7). The ExprTarget database (20), which was constructed to integrate various bioinformatic prediction tools along with our Exiqon and exon array experimental data, was queried to identify the negative correlation relationships between the miRNAs and mRNAs of interest (both 0.05 (for screening) and $10^{-4}$ (FDR<0.05, for multiple testing correction (21)) cut off were examined). Furthermore, the relationship between mRNA expression levels and sensitivity to carboplatin or cisplatin were queried through PACdb (19), another publicly available database we constructed to host results of SNP associations with chemotherapeutic agent sensitivity or with gene expression. Linear regression analysis was performed between miRNA expression and log$_2$-transformed carboplatin or cisplatin IC$_{50}$ with p<0.05 as cutoff. Linear regression was also used to evaluate the SNP-miRNA-mRNA-platinum sensitivity relationships in the replication samples with p<0.05 used as cut-off. In the discovery-phase samples, the nominal threshold p<0.05 was used to filter for relationships (among the SNP, miRNAs, and related mRNAs) to carry forward for replication and for additional functional studies.

**Functional annotation enrichment analyses.** We evaluated the Pearson correlation coefficient between gene pairs for those genes whose expression levels are correlated with miR-193b* and with sensitivity to both platinating agents. We found an enrichment of highly correlated genes (in particular, positively correlated genes) compared to the genomic background, which was derived from 1000 randomly selected pairs of genes from the genome (Supplemental Fig 1). This finding suggests that genes associated with both miR-193b* and platinum sensitivity may share the same biological pathway. Additionally, we performed functional annotation enrichment analyses using
DAVID (22, 23) with 3 lists of genes. First (level 1), we examined the 39 target genes for the eQTL rs1649942 in order to reveal novel pathways important for cellular sensitivity to both platinum agents. Secondly (level 2), we considered all potential gene targets of miR-193b* as predicted by ExprTarget (20) (668 from miRanda and 512 from the LCL-based expression data) and performed functional enrichment analysis to reveal pathways affected by a specific miRNA (miR-193b*). Finally (level 3), we performed pathway analysis on the 10 genes whose expression levels are negatively correlated with miR-193b* and also correlated with sensitivity to both platinating agents. We report functional terms that meet the nominal threshold p<0.05.

**Role of candidate miRNAs in platinum sensitivity in NCI60 tumor samples.** The expression of the miRNAs and –log10platinum GI50 data from the NCI60 data set were queried using CellMiner (24). Linear regression analysis was performed between miR-193b* expression (quantified using Agilent Human miRNA (V2)) and -log10GI50 of either platinum agent, independently for all 60 NCI60 samples as well as for a subset of 7 ovarian cancer cell lines.

**Functional validation for miR-193b*, its targets and platinum sensitivity.** To functionally validate the role of miRNAs in regulating gene expression and in conferring platinum sensitivity, we conducted miRNA over-expression experiments for miR-193b* in 2 randomly selected HapMap CEU samples (GM07055 and GM07056) as well as in an ovarian cancer cell line (OVCAR-3). Specifically, miRNA mimic (cat. #MSY0004767) for miR-193b* and scrambled control (AllStars Negative control, cat. #1027292) were purchased from Qiagen. Detailed methods of the over-expression and inhibition experiments in LCLs were described previously (17). In OVCAR-3, miR-193b* mimic
was transfected in using DharmaFECT 3 transfection reagent with existing Dharmacon DharmaFECT General Transfection protocol. miR-193b* and the gene expression levels of its targets were quantified through qPCR 5, 24, and 48 hours post transfection. A student t-test was performed between mimic treatment and controls at each time point. Cellular sensitivity to both carboplatin and cisplatin was measured using the method described above 24 hours post miR-193b* mimic transfection in LCLs and with CellTiter-Glo luminescent cell viability assay (Qiagen) 48 hours after mimic transfection in OVCAR-3. Cellular growth inhibition curve was plotted after increasing concentrations of carboplatin or cisplatin treatment. Two-way ANOVA was conducted to compare the cellular growth inhibition effect by platinum after treating the cells with either miR-193b* mimic or scramble control. P<0.05 was used to define statistical significance.
Results

**miRNAs associated with rs1649942 genotype play a role in platinum sensitivity via their effects on target gene expression.** We found that rs1649942 is associated (p<0.05) with 89 miRNA expression phenotypes (out of 201 tested) in the HapMap CEU lymphoblastoid cell lines (LCLs) discovery-phase samples (see Materials and Methods). Examining the relationship between these miRNAs and the 39 expression targets (mRNAs) of the same SNP (7), we selected for downstream analysis 61 of these miRNAs, for which higher expression resulted in reduced expression of at least one target gene (at nominal p<0.05) of rs1649942. There were 27 such target genes (Fig 1 left branch using a nominal threshold for screening).

When a miRNA or mRNA is nominally associated (p<0.05) with the IC50 of both carboplatin and cisplatin, we refer to the miRNA or mRNA as “platinum-associated”. Of the selected 27 expression targets of rs1649942, 14 (52%) were found to be platinum-associated. Three of the 61 miRNAs are platinum-associated and, in addition, negatively correlated (at nominal p<0.05) with at least one of the 14 platinum-associated target genes of rs1649942 (Fig 1 left branch). These 3 miRNAs are miR-193b*, miR-320a and miR-424. A summary of these SNP-miRNA-mRNA-platinum sensitivity relationships can be found in Table 1.

For multiple testing correction (on the miRNA-mRNA inverse relationships), we used an FDR approach (21). At FDR<0.05 (corresponding to p<10^-4) for the (negative) correlation between rs1649942-associated miRNAs and mRNAs, we found that 2 miRNAs (namely, the platinum-associated miR-193b* and miR-320a) are significantly
correlated with 5 platinum-associated genes (CRIM1, IFIT2, OAS1, KCNMA1 and GRAMD1B) (bold data in Table 1, Fig 1 right branch and Supplemental Fig 2).

To replicate these findings, we utilized 58 unrelated HapMap CEU III samples, for which the rs1649942 genotype and data on cellular sensitivity to platinum information are publicly available (18, 19). Indeed, the SNP was found to be significantly or suggestively associated with platinum sensitivity in these replication samples (p=0.03 and 0.07 for carboplatin and cisplatin IC$_{50}$, respectively). Note that the additional G allele is associated with platinum resistance in both the discovery-phase and replication samples. We also quantified the expression of miR-193b* and miR-320a along with their 5 putative binding targets in these samples using qPCR method. The expression of miR-193b* was found to be platinum-associated in the replication samples (p=0.03 and 0.02, for carboplatin and cisplatin respectively. See Fig 2). Increased expression level of miR-193b* conferred greater platinum resistance. Furthermore, we replicated the negative correlation between miR-193b* and two of its targets (CRIM1 and IFIT2. Fig 3). These 2 genes, along with GRAMD1B and KCNMA1 (Table 2) (which were identified to correlate with the expression of miR-320a and with miR-424 to a lesser degree), were found to be platinum-associated in the separate (replication-phase) dataset just as in the discovery-phase samples. On the other hand, we did not replicate the relationship between miR-320a expression and platinum sensitivity.

**Functional annotation enrichment analyses.** Using DAVID, we found enrichment of certain Biological Functional (BF) annotation terms such as *immune response* from the level 2 analysis regardless of the method used to obtain the miR-193b* target genes (enrichment p value= 0.01 and 0.00001 for predicted target genes derived...
using miRanda and the genome-wide gene expression datasets in LCLs, respectively) and from the level 3 analysis (p=0.046 using the ten miR-193b* and platinum sensitivity-related genes).

**Functional validation of the role of miR-193b* in platinum sensitivity.** Using two randomly selected CEU LCLs treated with miR-193b* mimic, we found that the addition of miRNA mimic yields higher intracellular miR-193b* expression at 5, 24, and 48 hours post transfection in both cell lines. This increase in miR-193b* expression also led to decreased expression of **CRIM1** at 5 hours post transfection. We did not observe decreased expression of **IFIT2** at these time points. More importantly, cells became more resistant to both carboplatin and cisplatin treatment when compared to treating them with scramble control (p<0.0001 with two-away ANOVA for both drugs. Fig 4A and 4B).

**Role of miR-193b* in cancer cell lines.** We queried the NCI60 dataset through CellMiner and found that increased miR-193b* expression conferred greater platinum resistance in 7 ovarian cancer cell lines (Supplemental Fig 3). Furthermore, we conducted miR-193b* over-expression experiments in an OVCAR-3 ovarian cancer cell line. The addition of miR-193b* mimic (over-expression) resulted in increased expression of miR-193b* (compared to control, t-test p<0.0001), decreased expression of **CRIM1** (compared to control, t-test p=0.07 at 24 hours and p=0.02 at 48 hours) and increased cellular resistance to carboplatin in OVCAR-3 cell line (p<0.0001 with two away ANOVA. Fig 4C).
Discussion

By triangulating a SNP (that has been shown to be associated with carboplatin and cisplatin sensitivity in lymphoblastoid cell lines and in patient samples) with miRNAs and their putative mRNA targets, we identified the potential role of miR-193b*, miR-320a and miR-424 in platinum sensitivity. Replication and over-expression experiments confirmed the role of increased miR-193b* expression in conferring platinum resistance in both LCLs and cancer cell lines. Our study integrated genetic variation, miRNA expression, and transcriptome expression to characterize inter-individual variation in cellular sensitivity to platinating agents. The identified miRNAs and genes may serve as potential targets for platinum resistance.

miR-193b has been shown to play an important role in several types of cancers, acting like a tumor suppressor in some (25-27), while demonstrating over-expression in others (28). miR-193b has also been found to down-regulate estrogen receptor alpha in breast cancer cell lines (29). More recently, a SNP in the miR-193b precursor flanking region (rs30236) was found to potentially associate with non-small cell lung cancer survival, a disease that is commonly treated with platinum agents (30).

In our study, the expression of miR-193b*, a miRNA on the opposite arm of miR-193b of the same pre-miRNA hairpin structure, along with 2 of its targets (CRIM1 and IFIT2) were shown to be platinum-associated in the HapMap CEU discovery and replication samples. Checking publicly available drug-induced cellular sensitivity phenotype data (19), including etoposide and daunorubicin, the effect of miR-193b* and the 2 targets was found to be specific to the platinum agents. In addition, over-expression of miR-193b* resulted in decreased CRIM1 expression and increased carboplatin and
cisplatin resistance in LCLs. This supports the role of miR-193b* in platinum sensitivity. Furthermore, higher miR-193b* expression is observed to result in greater platinum resistance in 7 ovarian cancer cell lines that are a part of the NCI60 dataset. Our miR-193b* over-expression experiment in OVCAR-3 demonstrates that increased miR-193b* expression in this ovarian cancer cell line results in increased resistance to carboplatin.

CRIM1 encodes cysteine rich transmembrane BMP regulator 1 (chordin-like) protein. The encoded protein may play a role in tissue development though interactions with members of the transforming growth factor beta family, such as bone morphogenetic proteins (31). Various miRNAs have shown their regulative effect on CRIM1 expression, resulting in diverse biological functions. For example, five down-regulated miRNAs were shown to subsequently affect CRIM1 protein expression and affect neuronal lineage differentiation in unrestricted somatic stem cells and (32). miR-20b was found to enhance osteogenesis by repressing CRIM1 in human mesenchymal stem cells (hMSCs) (33). Unfortunately, miR-193b* was not among those miRNAs evaluated in these studies. Interestingly, a recent study demonstrated the role of CRIM1 in drug resistance in myeloid leukemia cell lines (34). Another target of miR-193b*, IFIT2 (interferon-induced protein with tetratricopeptide repeats 2), whose expression is also correlated with platinum sensitivity in our study, was recently reported to induce apoptosis via a mitochondrial pathway (35). Furthermore, platinum-induced cellular apoptosis was also reported to be a useful pharmacogenomic trait (36).

In our study, two of miR-320a’s targets (namely, GRAMD1B and KCNMA1) were found to correlate in expression with platinum sensitivity in both the discovery and replication samples; however, we did not replicate the role of miR-320a in platinum
sensitivity. Since various other miRNAs have shown correlation with these two genes (e.g., miR-424), it is possible that these genes may be affected by other miRNAs which directly or indirectly affect platinum sensitivity. Interestingly, these 2 gene expression-drug sensitivity relationships are not unique to the platinums, with observed relationships to etoposide and daunorubicin as well.

In summary, a genetic variation that predicts platinum sensitivity led to the discovery of the role of miR-193b* in conferring platinum sensitivity. Our data support that increased expression of miR-193b* and the subsequent effect on this miRNA’s gene targets contribute to variability in platinum resistance.

Acknowledgements

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References:


Table 1. The relationships among rs1649942, miRNAs, mRNAs and cellular sensitivity to platinum agents in the HapMap CEU discovery samples.

<table>
<thead>
<tr>
<th>rs1649942 association with platinum IC$_{50}$</th>
<th>miRNA P value</th>
<th>rs1649942-miR association p value</th>
<th>miRNA P value association with platinum IC$_{50}$</th>
<th>gene</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>carboplatin</td>
<td></td>
<td></td>
<td></td>
<td>Crim1</td>
<td>3E-5</td>
</tr>
<tr>
<td>cisplatin</td>
<td>1E-5</td>
<td>3.4E-3</td>
<td>7.3E-3</td>
<td>1.6E-2</td>
<td></td>
</tr>
<tr>
<td><strong>miR-193b</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>miR-320a</strong></td>
<td>4.5E-2</td>
<td>1.0E-2</td>
<td>3.7E-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>miR-424</strong></td>
<td>6.7E-3</td>
<td>2.4E-2</td>
<td>2.7E-2</td>
<td></td>
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</tr>
<tr>
<td><strong>Bold miRNA and gene pairs meet negative correlation and p&lt;10^-4 cutoff and were evaluated in replication samples.</strong></td>
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</tr>
</tbody>
</table>
Table 2. The relationships between miRNA and gene expression as well as between gene expression and platinum sensitivity in HapMap CEU replication samples.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>P values between miRNA and gene</th>
<th>P values and direction of relationship</th>
<th>Whether direction agreed with the discovery phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carboplatin IC₅₀ (direction)</td>
<td>Cisplatin IC₅₀ (direction)</td>
</tr>
<tr>
<td>miR-193b*</td>
<td>1.8E-2  CRIM1</td>
<td>8.4E-2 (-)</td>
<td>4.6E-2 (-)</td>
</tr>
<tr>
<td>miR-320a</td>
<td>7.0E-3  IFIT2</td>
<td>3.6E-2 (-)</td>
<td>1.7E-3 (-)</td>
</tr>
<tr>
<td></td>
<td>5.9E-1  OAS1</td>
<td>1.0E-1 (-)</td>
<td>1.5E-1 (-)</td>
</tr>
<tr>
<td></td>
<td>8.7E-1  GRAMD1B</td>
<td>1.1E-3 (+)</td>
<td>2.1E-3 (+)</td>
</tr>
<tr>
<td></td>
<td>2.0E-1  KCNMA1</td>
<td>4.6E-3 (+)</td>
<td>3.0E-3 (+)</td>
</tr>
</tbody>
</table>

“-“ indicates that increased gene expression resulted in decreased drug IC₅₀, suggesting greater sensitivity. Vice versa for “+” direction.

Bold p values meet the p<0.05 threshold in replication samples.
Figure Legends

Figure 1. Overall workflow and findings in the discovery-phase samples.

Figure 2. Discovered and replicated positive correlation between miR-193b* expression and platinum sensitivity. A, B) The relationship between miR-193b* expression on the one hand and carboplatin and cisplatin IC\textsubscript{50} on the other in the discovery-phase CEU samples, respectively; C, D) The relationship between miR-193b* expression on the one hand and carboplatin and cisplatin IC\textsubscript{50} on the other in the replication CEU samples, respectively. Exiqon arrays were used to obtain miRNA expression data in the discovery-phase samples while real-time PCR method was used to quantify miRNA expression in the replication samples.

Figure 3. Discovered and replicated negative correlation in expression between miR-193b* and two of its targets. A, B) The expression relationship between miR-193b* on the one hand and \textit{CRIM1} and \textit{IFIT2} on the other in the discovery-phase CEU samples, respectively; C, D) The expression relationship between miR-193b* on the one hand and \textit{CRIM1} and \textit{IFIT2} on the other in the replication CEU samples, respectively. Exiqon and exon arrays were used to obtain miRNA and mRNA expression data in discovery-phase samples while real-time PCR method was used to quantify miRNA and mRNA expression in the replication samples.

Figure 4. Effect of miRNA mimic on platinum sensitivity. A) miR-193b* mimic effect on cellular sensitivity to carboplatin 24 hours post transfection. Data shown in this figure is
derived from a randomly selected LCL samples (GM07056). B) miR-193b* mimic effect on cellular sensitivity to cisplatin 24 hours post transfection. Data shown in this figure is derived from a randomly selected LCL samples (GM07056). C) miR-193b* over-expression effect on carboplatin sensitivity in an OVCAR-3 cell line 48 hours post transfection. Dash line represents the growth inhibition curve after transfecting cells with miR-193b* mimic while the solid line represents the growth inhibition curve of the same cell line treated with a scramble control. A two-way ANOVA method was used to compare the cellular growth inhibition effect by platinum after treating the cells with either miR-193b* mimic or scramble control.
Fig 1.

rs1649942 and 201 miRNA expression

SNP and miRNA association (p<0.05)

89 miRNAs

Negative correlation with 39 rs1649942 related gene expression

P<0.05

FDR<0.05; P<0.0001

61 miRNAs negatively correlated with 27 mRNA expression

14 miRNAs negatively correlated with 10 mRNA expression

Correlation with both carboplatin and cisplatin IC_{50} (p<0.05)

3 platinum-associated miRNAs and 14 platinum-associated mRNAs

2 platinum-associated miRNAs and 5 platinum-associated mRNAs

Nominal threshold screening

Multiple testing approach
Fig 2.
Fig 3.
Fig 4.
Molecular Cancer Therapeutics

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