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

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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.

Keywords

microRNA; gene expression; platinum; HapMap; SNP

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

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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 Reseach (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 OVCAR-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 $-\log_{10}$ platinum GI_{50} 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 $-\log_{10}GI_{50}$ 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 IC_{50} 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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

References

1. Williams C, Whitehouse J. Cis-platinum: a new anticancer agent. 1979; 1:6179.
2. Huang RS, Duan S, Kistner EO, Christine HM, Dolan ME. Genetic Variants Assoicated with Carboplatin-induced Cytotoxicity in Cell Lines derived from Africans Molecular Cancer Therapeutics. 2008; 7:3038–46.
3. Huang RS, Duan S, Shukla SJ, Kistner EO, Clark TA, Chen TX, et al. Identification of Genetic Variants Contributing to Cisplatin-Induced Cytotoxicity using a Genome-wide Approach. Am J Hum Genet. 2007; 81:427–37. [PubMed: 17701890]
4. Gamazon E, Huang R, Dolan M, Cox N. Copy number polymorphisms and anticancer pharmacogenomics. Genome Biol. 2011; 12:R46. [PubMed: 21609475]
5. Huang RS, Johnatty SE, Gamazon ER, Im HK, Ziliak D, Duan S, et al. Platinum Sensitivity–Related Germline Polymorphism Discovered via a Cell-Based Approach and Analysis of Its Association with Outcome in Ovarian Cancer Patients. Clinical Cancer Research. 2011; 17:5490–500. [PubMed: 21705454]

6. Ziliak D, O'Donnell PH, Im HK, Gamazon ER, Chen P, Delaney S, et al. Germline polymorphisms discovered via a cell-based, genome-wide approach predict platinum response in head and neck cancers. Translational Research. 2011; 157:265–72. [PubMed: 21497773]
7. Gamazon ER, Huang RS, Cox NJ, Dolan ME. Chemotherapeutic drug susceptibility associated SNPs are enriched in expression quantitative trait loci. Proceedings of the National Academy of Sciences. 2010; 107:9287–92.
8. Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk R, Cuppen E. Phylogenetic shadowing and computational identification of human microRNA genes. Cell. 2005; 120:21–4. [PubMed: 15652478]
9. Lewis B, Burge C, Bartel D. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005; 120:15–20. [PubMed: 15652477]
10. Chitwood D, Timmermans M. Small RNAs are on the move. Nature. 2010; 467:415–9. [PubMed: 20864994]
11. Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, Boehm M, et al. MicroRNAs as Potential Agents to Alter Resistance to Cytotoxic Anticancer Therapy. Cancer Research. 2007; 67:11111–6. [PubMed: 18056433]
12. The International HapMap Project. Nature. 2003; 426:789–96. [PubMed: 14685227]
13. A map of human genome variation from population-scale sequencing. Nature. 2010; 467:1061–73. [PubMed: 20981092]
14. Huang RS, Kistner EO, Bleibel WK, Shukla SJ, Dolan ME. Effect of population and gender on chemotherapeutic agent-induced cytotoxicity. Molecular Cancer Therapeutics. 2007; 6:31–6. [PubMed: 17237264]
15. Zhang W, Duan S, Kistner EO, Bleibel WK, Huang RS, Clark TA, et al. Evaluation of Genetic Variation Contributing to Differences in Gene Expression between Populations. The American Journal of Human Genetics. 2008; 82:631–40.
16. Gamazon ER, Ziliak D, Im HK, LaCroix B, Park D, Cox NJ, et al. Genetic Architecture of microRNA Expression: Implications for the Transcriptome and Complex Traits. Am J Hum Genet. 2012 In press.
17. Huang RS, Gamazon ER, Ziliak D, Wen Y, Im HK, Zhang W, et al. Population differences in microRNA expression and biological implications. RNA Biology. 2011; 8 Epub ahead of print.
18. Wheeler H, Gamazon E, Stark A, O'Donnell P, Gorsic L, Huang R, et al. Genome-wide meta-analysis identifies variants associated with platinating agent susceptibility across populations. Pharmacogenomics J. 2011 Epub ahead of print.
19. Gamazon E, Duan S, Zhang W, Huang R, Kistner E, Dolan M, et al. PACdb: a database for cell-based pharmacogenomics. Pharmacogenet Genomics. 2010; 20:269–73. [PubMed: 20216476]
20. Gamazon ER, Im HK, Duan S, Lussier YA, Cox NJ, Dolan ME. ExprTarget: An integrative approach to predicting human microRNA targets. PLoS One. 2010; 5:e13534. [PubMed: 20975837]
21. Storey JD, Tibshirani R. Statistical significance for genome-wide studies. Proc Natl Acad Sci USA. 2003; 100:9440–5. [PubMed: 12883005]
22. Huang D, Sherman B, Lempicki R. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009; 4:44–57. [PubMed: 19131956]
23. Huang D, Sherman B, Lempicki R. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009; 37:1–13. [PubMed: 19033363]
24. Shankavaram U, Varma S, Kane D, Sunshine M, Chary K, Reinhold W, et al. CellMiner: a relational database and query tool for the NCI-60 cancer cell lines. BMC Genomics. 2009; 10:277. [PubMed: 19549304]
25. Chen J, Feilotter HE, Paré GC, Zhang X, Pemberton JGW, Garady C, et al. MicroRNA-193b Represses Cell Proliferation and Regulates Cyclin D1 in Melanoma. The American Journal of Pathology. 2010; 176:2520–9. [PubMed: 20304954]
26. Li XF, Yan PJ, Shao ZM. Downregulation of miR-193b contributes to enhance urokinase-type plasminogen activator (uPA) expression and tumor progression and invasion in human breast cancer. Oncogene. 2009; 28:3937–48. [PubMed: 19701247]

27. Rauhala H, Jalava S, Isotalo J, Bracken H, Lehmusvaara S, Tammela T, et al. miR-193b is an epigenetically regulated putative tumor suppressor in prostate cancer. Int J Cancer. 2010; 127:1363–72. [PubMed: 20073067]

28. Unno K, Zhou Y, Zimmerman T, Platanias L, Wickrema A. Identification of a novel microRNA cluster miR-193b-365 in multiple myeloma. Leuk Lymphoma. 2009; 50:1865–71. [PubMed: 19883314]

29. Leivonen SK, Makela R, Ostling P, Kohonen P, Haapa-Paananen S, Kleivi K, et al. Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. Oncogene. 2009; 28:3926–36. [PubMed: 19684618]

30. Hu Z, Shu Y, Chen Y, Chen J, Dong J, Liu Y, et al. Genetic Polymorphisms in the Precursor MicroRNA Flanking Region and Non-Small Cell Lung Cancer Survival. Am J Respir Crit Care Med. 2011; 183:641–8. [PubMed: 20889907]

31. Wilkinson L, Kolle G, Wen D, Piper M, Scott J, Little M. CRIM1 regulates the rate of processing and delivery of bone morphogenetic proteins to the cell surface. J Biol Chem. 2003; 278:34181–8. [PubMed: 12805376]

32. Iwaniuk K, Schira J, Weinhold S, Jung M, Adjaye J, Müller H, et al. Network-Like Impact of MicroRNAs on Neuronal Lineage Differentiation of Unrestricted Somatic Stem Cells from Human Cord Blood. Stem Cells Dev. 2011; 20:1383–94. [PubMed: 21067317]

33. He J, Zhang J, Yi C, Lv Q, Xie W, Li J, et al. miRNA-mediated functional changes through co-regulating function related genes. PLoS One. 2010; 5:e13558. [PubMed: 21042576]

34. Prenkert M, Uggla B, Tidefelt U, Strid H. CRIM1 is expressed at higher levels in drug-resistant than in drug-sensitive myeloid leukemia HL60 cells. Anticancer Res. 2010; 30:4157–61. [PubMed: 21036735]

35. Stawowczyk M, Van Scoy S, Kumar K, Reich N. The interferon stimulated gene 54 promotes apoptosis. J Biol Chem. 2011; 286:7257–66. [PubMed: 21190939]

36. Wen Y, Gorsic L, Wheeler H, Ziliak D, Huang R, Dolan M. Chemotherapeutic-induced apoptosis: a phenotype for pharmacogenomics studies. Pharmacogenet Genomics. 2011; 21:476–88. [PubMed: 21642893]

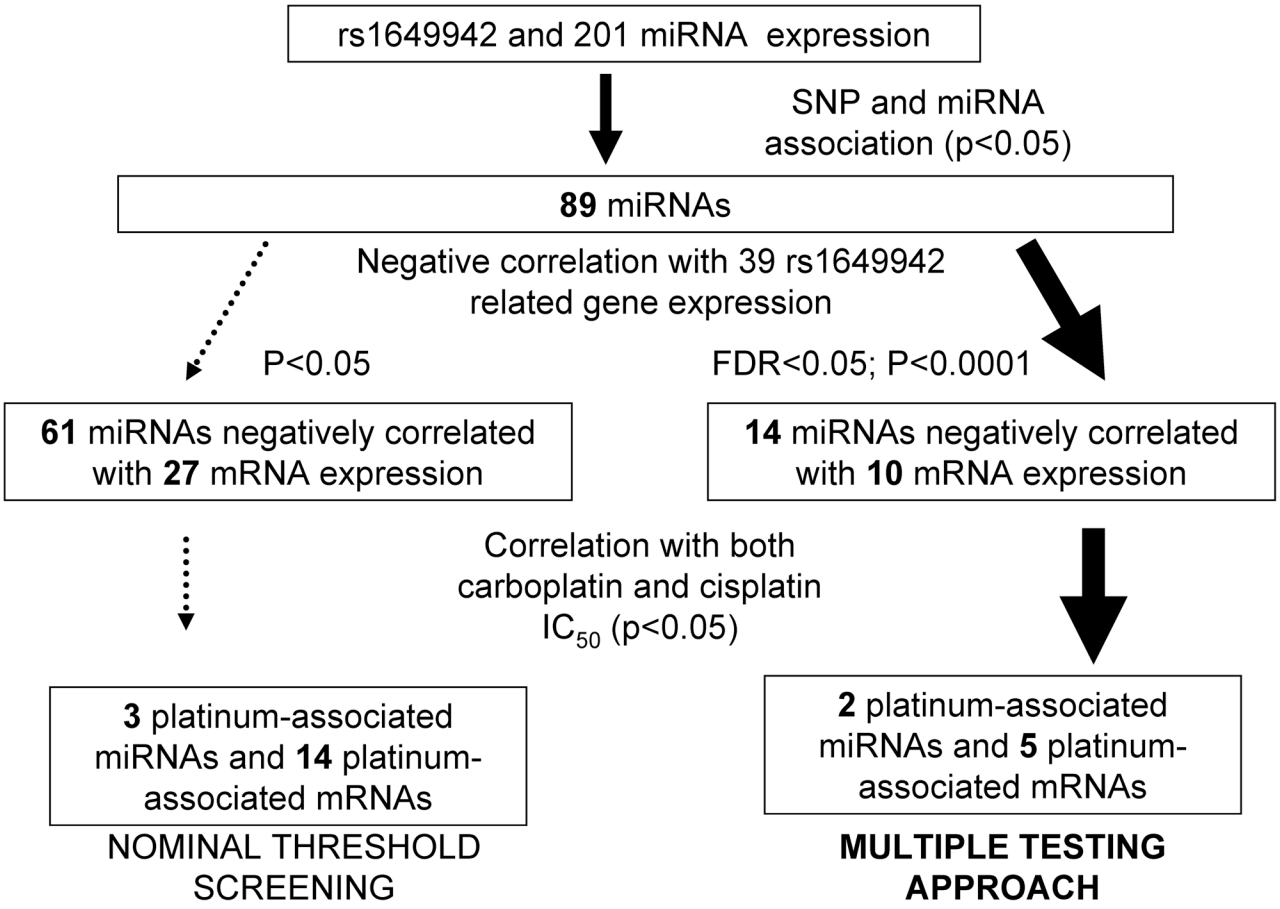

Figure 1.
Overall workflow and findings in the discovery-phase samples.

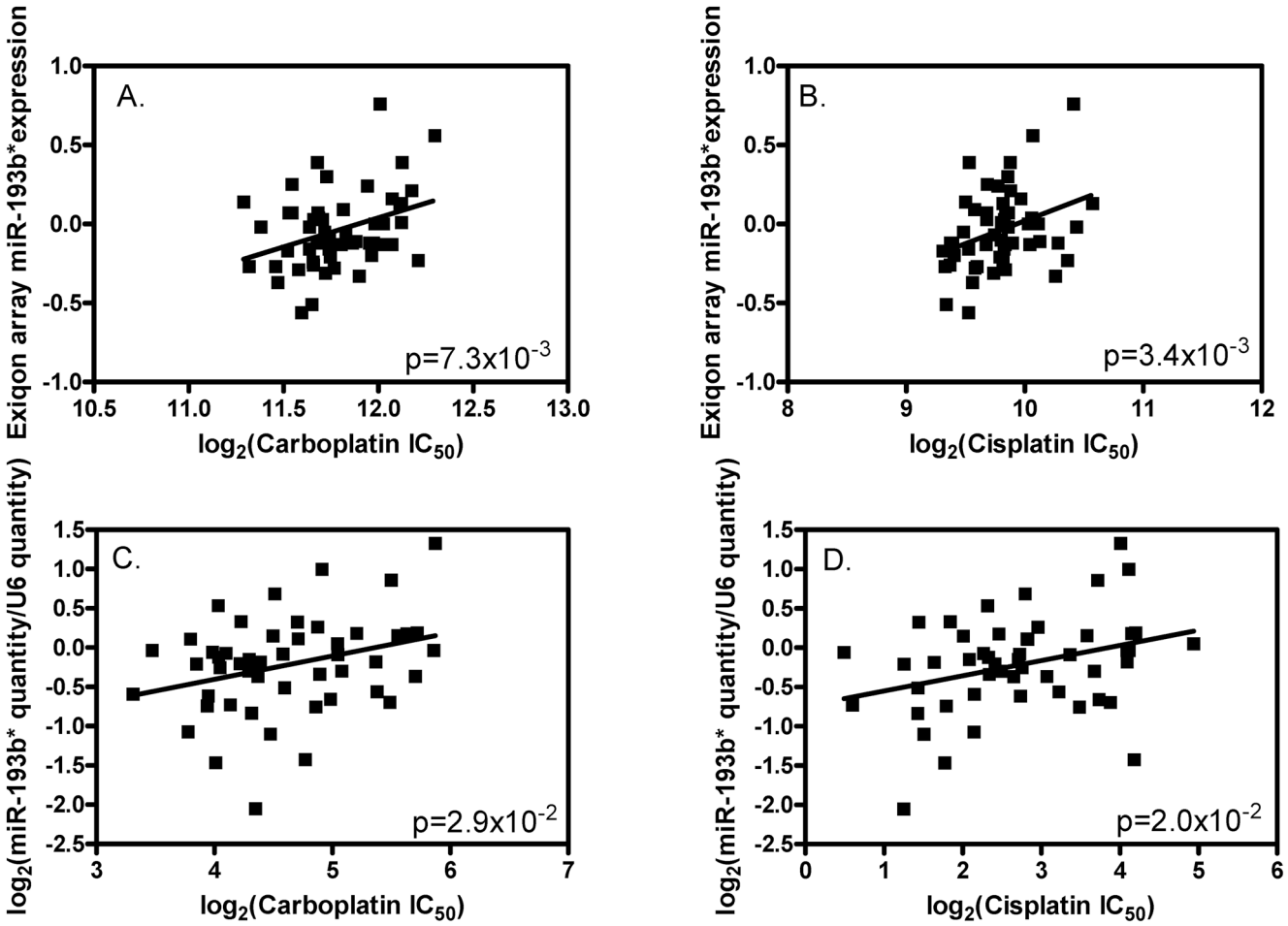

Figure 2.

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_{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_{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.

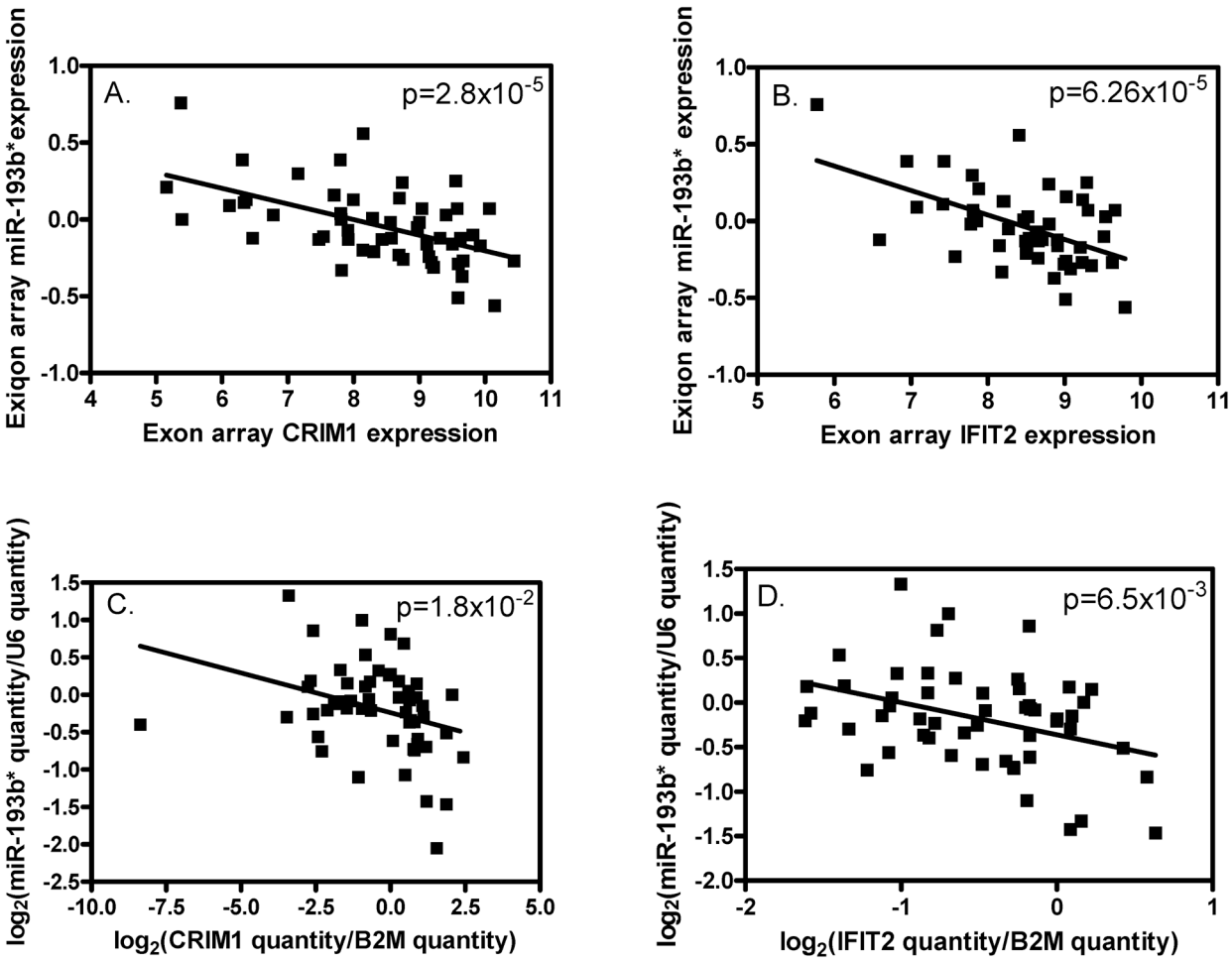

Figure 3.

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 *CRIM1* and *IFIT2* on the other in the discovery-phase CEU samples, respectively; C, D) The expression relationship between miR-193b* on the one hand and *CRIM1* and *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.

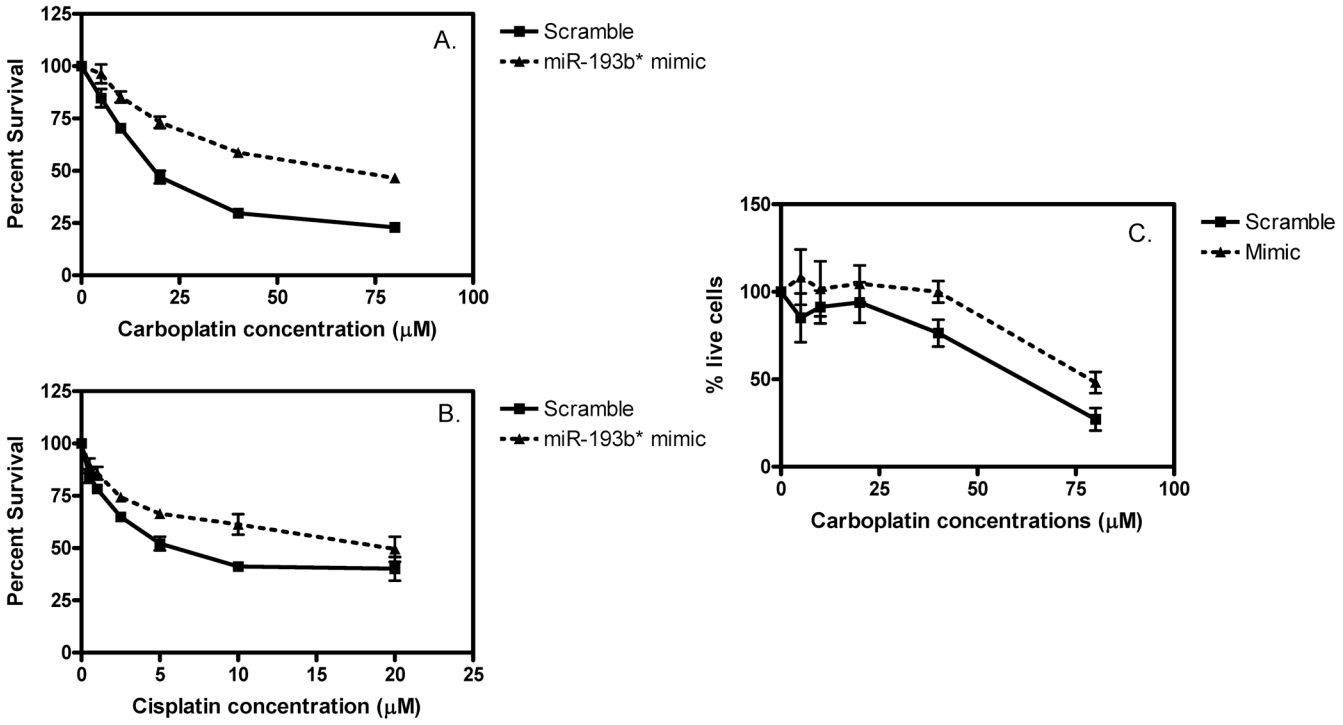

Figure 4.

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.

Table 1

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

rs1649942 association with platinum IC_{50}P value		miRNA	rs1649942-miR association p value	miRNA P value association with platinum IC_{50}		gene	P values			
carboplatin	cisplatin			Carboplatin	cisplatin		rs16499 42-gene	Gene-carboplatin	Gene-cisplatin	miRNA-gene
1E-5	7E-5	**miR-193b***	3.4E-3	7.3E-3	1.6E-2	***CRIM1***	3E-5	1.0E-4	9.8E-4	2.8E-5
						IFIT2	1E-4	1.8E-2	7.6E-3	6.3E-5
						OAS1	1E-5	3.7E-2	4.3E-2	5.2E-5
						CCR6	5E-5	3.3E-2	2.0E-2	3.1E-4
								3.5E-4	1.5E-4	
						CYP2R1	1E-4	3.3E-4	2.5E-3	2.0E-2
							1E-4	2.2E-5	1.9E-3	2.7E-3
						KYNU	9E-6	9.3E-4	2.7E-4	4.8E-3
						RAPGEF5	2E-6	2.0E-2	4.6E-2	1.0E-2
						SLC2A5	2E-5	6.1E-3	1.5E-3	6.6E-3
						USP12	9E-5	5.1E-4	4.2E-4	1.3E-3
						ZHX12	7E-5	1.2E-5	6.2E-5	2.0E-2
		miR-320a	4.5E-2	1.0E-2	3.7E-2	***GRAMD1B***	1E-4	4.7E-4	4.4E-3	3.5E-5
						KCNMA1	7E-5	2.4E-2	3.4E-2	9.1E-5
						PSTPIP2	2E-5	1.6E-4	7.1E-4	1.9E-3
		miR-424	6.7E-3	2.4E-2	2.7E-2	*GRAMD1B*	1E-4	4.7E-4	4.4E-3	5.9E-3
						KCNMA1	7E-5	2.4E-2	3.4E-2	9.8E-3
						PSTPIP2	2E-5	1.6E-4	7.1E-4	2.0E-2

rs1649942 association with platinum IC_{50}P value		miRNA	rs1649942-miR association p value	miRNA P value association with platinum IC_{50}		gene	P values			
carboplatin	cisplatin			Carboplatin	cisplatin		rs16499 42-gene	Gene-carboplatin	Gene-cisplatin	miRNA-gene
						SHFM3P1	2E-6	2.8E-3	1.2E-2	4.0E-2
							4E-5			

Bold miRNA and gene pairs meet negative correlation and $p<10^{-4}$ cutoff and were evaluated in replication samples.

Table 2

The relationships between miRNA and gene expression as well as between gene expression and platinum sensitivity in HapMap CEU replication samples.

miRNA	P values between miRNA and gene	gene	P values and direction of relationship		Whether direction agreed with the discovery phase	
			Carboplatin IC_{50} (direction)	Cisplatin IC_{50} (direction)	carboplatin	cisplatin
*miR-193b**	**1.8E-2**	*CRIM1*	8.4E-2 (−)	**4.6E-2** (−)	Y	Y
	7.0E-3	*IFIT2*	**3.6E-2** (−)	**1.7E-3** (−)	Y	Y
	5.9E-1	*OAS1*	1.0E-1 (−)	1.5E-1 (−)	Y	Y
miR-320a	8,7E-1	*GRAMD1B*	**1.1E-3** (+)	**2.1E-3** (+)	Y	Y
	2.0E-1	*KCNMA1*	**4.6E-3** (+)	**3.0E-3** (+)	Y	Y

"−" indicates that increased gene expression resulted in decreased drug IC_{50}, suggesting greater sensitivity. Vice versa for "+" direction.

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