Analysis of chemotherapy response programs in ovarian cancers by the next generation sequencing technologies

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Abstract

Objective—To understand the chemotherapy response program in ovarian cancer cells at deep transcript sequencing levels.

Methods—Two next-generation sequencing technologies—MPSS (massively parallel signature sequencing) and SBS (sequencing by synthesis) — were used to sequence the transcripts of IGROV1 and IGROV1-CP cells, and to sequence the transcripts of a highly chemotherapy responsive and a highly chemotherapy resistant ovarian cancer tissue.

Results—We identified 3,422 signatures (2957 genes) that are significantly different between IGROV1 and IGROV1-CP cells (P <0.001). Gene Ontology (GO) term GO:0001837 (epithelial to mesenchymal transition) and GO:0034330 (cell junction assembly and maintenance) are enriched in genes that are over expressed in IGROV1-CP cells while apoptosis related GO terms are enriched in genes over expressed in IGROV1 cells. We identified 1,187 tags (corresponding to 1,040 genes) that are differentially expressed between the chemotherapy responsive and the persistently chemotherapy resistant ovarian cancer tissues. GO term GO:0050673 (epithelial cell proliferation) and GO:0050678 (regulation of epithelial cell proliferation) are enriched in the genes over expressed in the chemotherapy resistant tissue while the GO:0007229 (integrin-mediated signaling pathway) is enriched in the genes over expressed in the chemotherapy sensitive tissue. An integrative analysis identified 111 common differentially expressed genes including two bone morphogenetic proteins

(BMP4 and BMP7), six solute carrier proteins (SLC10A3, SLC16A3, SLC25A1, SLC35B3, SLC7A5 and SLC7A7), transcription factor POU5F1 (POU class 5 homeobox 1), and KLK10 (kallikrein-related peptidase 10). A network analysis revealed a subnetwork with three gene BMP7, NR2F2 and AP2B1 that were consistently over expressed in the chemoresistant tissue or cells compared to the chemosensitive tissue or cells.

Conclusion—Our database offers the first comprehensive view of the digital transcriptomes of ovarian cancer cell lines and tissues with different chemotherapy response phenotypes.

Introduction

Ovarian cancer ranks fourth in cancer mortality among women in the United States with an estimated 14,600 deaths and more than 21,550 new cases in 2009 (American Cancer Society Statistics for 2009). Ovarian carcinoma has remained the most challenging of all the gynecologic malignancies for two reasons. First, early-stage ovarian cancer that has a far better prognosis than late-stage ovarian cancer cannot be easily detected [1]. Early state (Stage I or II) ovarian cancers have a 5-year survival rate of 90% (stage I) or 70% (stage II) whereas late state cancers (Stage III and IV) have only about a 20% 5-year survival rate [2,3]. Second, standard chemotherapy approaches such as a combination of paclitaxel and carboplatin often fail [2,3]. Why chemotherapy fails is not completely understood. We hypothesize that a complete understanding of chemotherapy response requires a systems approach to define the pathways involved in chemotherapy response and to understand how genes in pathways work together by systematic perturbation followed by the observation on the effects of perturbation. A systems approach to chemotherapy responsiveness of ovarian cancer can identify key nodal points in pathways that differ between chemotherapy sensitive and chemotherapy resistant cells. Intervening at the key nodal points will give us the ability to develop novel therapeutic approaches.

A systems approach starts with the delineation of the parts list of a system. The most sensitive technology around today to delineate the parts lists of transcriptomes of cells is applying the next-generation sequencing technologies. In order to study chemotherapy response we have applied two platforms of the next generation (next-gen) sequencing technologies--the massively parallel signature sequencing (MPSS) technology [4,5] and the sequence-by-synthesis (SBS) technology [6]. The next-gen sequencing technology allows identification and cataloging of almost all mRNAs, even those with only one or a few transcripts per cell. To map network perturbations in cancer initiation and progression, the changes in expression levels of virtually all transcripts must be measured. Differentially expressed genes thus identified can be mapped onto cellular networks to provide a systemic understanding of changes in the cellular state. Certain low-abundance transcripts, such as those encoding transcription factors and signal transducers, wield significant regulatory influences in spite of the fact that they may be present in the cell at very low copy numbers.

We present our analysis in ovarian cancer cells using the next-generation sequencing technologies to understand chemotherapy resistance. Platinum compounds, such as cis and carboplatin, are the most active ovarian cancer chemotherapy drug and the standard treatment for nearly all women diagnosed with ovarian cancer. IGROV-1 and IGROV-1-CP cells are good model systems to use to understand the mechanism of cisplatin resistance. IGROV-1-CP cells are cisplatin resistant ovarian cancer cells derived experimentally from cisplatin sensitive parental IGROV-1 cells after prolonged exposure to cisplatin compound and selection of resistant clones [7]. In additional to cell line model, we also applied the newly developed next-generation technology, named sequence by synthesis (SBS) technology, to sequence two ovarian cancer tissues with extreme sensitivity and extreme resistance to standard combination

chemotherapy of carboplatin and paclitaxel [8,9]. An integrative analysis allowed us to gain a better understanding of the chemotherapy resistance program.

Materials and Methods

Cells and tissues

The human ovarian carcinoma DDP-resistant cell line IGROV-1/CP was kindly provided by Dr. Stephen Howell's Lab at Moores UCSD Cancer Centre, San Diego, and grown at 37 °C in RPMI 1640 supplemented with 10% fetal bovine serum, 50 IU/ml penicillin and 50ug/ml streptomycin with a humidified atmosphere containing 5% CO2. Ovarian cancer tissues were obtained at the Department of Gynecological Oncology, Roswell Park Cancer Institute, with IRB approval and informed consent.

Next-generation sequencing analysis

For MPSS analysis, MPSS cDNA libraries were constructed, and individual cDNA sequences were amplified and attached to individual beads and sequenced as described elsewhere [4]. The resulting signatures, generally 17 bases in length, were annotated using the most recently annotated human genome sequence, and the human Unigene according to a previously published method [10]. We considered only 100% matches between an MPSS signature and a genome signature. We also excluded those signatures that expressed at less than 3 TPM in both IGROV-1 and IGROV1-CP libraries, as they might not be reliably detected [11]. Additionally, we classified MPSS signatures by their positions relative to polyadenylation signals and poly (A) tails and by their orientation relative to the $5' \rightarrow 3'$ orientation of source mRNA as we have previously described [5]. The Z-test [12] was used to calculate P values for comparison of gene expression levels between the cell lines. For SBS analysis, the library was prepared using the Solexa/Illumina's Gene Expression-Tag Profiling with DpnII and sequencing was carried out using the Solexa/Illumina 1 G genetic analyzer. We used the algorithm intensity-dependent selection of expression ratios (ISER)[13] (https://ipe.cbmeg.unicamp.br/pub/PMmA) to identify differentially expressed genes from the SBS data.

If a gene had multiple tags, we kept the information for each tag in the supplementary tables so as to capture alternative spliced and alternative polyadenylated forms. The tag with the most abundant expression (sum of the expression in TPM) was then used to represent the gene.

Gene Ontology analysis

High-Throughput GoMiner [14] was used to find statistically over represented Gene Ontology (GO) terms. The changed list was used as input, and the total list was auto-generated by the program. The root category used is the GO biological process, and the GO terms at level 3 categories were used for the analysis. Enriched GO terms with $\log 10(P) > 2$ were considered significant.

Processing of the Cancer Genome Atlas (TCGA)'s next-generation sequencing data and their comparisons to our data

The TCGA data on the next-generation sequencing were down loaded from its data portal (cancergenome.nih.gov/dataportal/). The sequence tags were aligned against cDNA database (GRCh37.55) from the Ensembl (www.ensembl.org/) using the SOAP2 (v2.19) program [15], which is an ultrafast short read alignment tool. Up to 1 mismatch was allowed during the alignment. If a tag was mapped to more than 2 genes, then it was filtered out. If a tag mapped to two genes, its counts was assigned to the more abundant gene. The count numbers of all mapped tags to a gene were summed to represent the expression abundance of the gene. The expression levels were normalized to one million to calculate the expression level in TPM

(transcript per million) for each gene. The comparisons of the TCGA's data to our data were performed at gene levels.

Results

MPSS analysis of IGROV-1 and IGROV1-CP cells

We conducted an MPSS analysis of 2.7 and 2.5 million signatures for the cisplatin sensitive IGROV1 cell line and its cisplatin resistant derivative IGROV1-CP cell line. The raw data for the MPSS dataset are available for download at http://www.zcni.zju.edu.cn/en/OV/download.html. We obtained 16,900 and 18, 938 reliable unique signatures for IGROV1 and IGROV1-CP cells respectively. Transcript tags with matches to a cDNA or EST sequence were further classified based on the signatures' relative orientation to the transcription direction and their position relative to a polyadenylation site and/or poly(A) tail. We first restricted our analysis to those MPSS signatures corresponding to cDNAs with poly(A) tails and/or polyadenylation sites, so that corresponding genes could be conclusively identified [5]. We used the *Z*-test [12] to identify differential gene expression. In the end, we obtained 3,422 signatures that were significantly different between IGROV1 and IGROV1-CPcells (P <0.001) (Supplementary Table 1). These tags represented 2,957 genes, suggesting that many of the genes have multiple tags, possibly representing alternative polyadenylated isoforms for which the MPSS technology is able to capture [5,10,11].

For those genes with multiple tags, we used the expression value of the most abundant tag to represent that gene. The top 20 over and under expressed genes are shown in Table 1. Interestingly, over expressed genes in the cisplatin resistant cell line IGROV1-CP include macrophage migration inhibitory factor (glycosylation-inhibiting factor), bone morphogenetic protein 7 (osteogenic protein 1), nuclear receptor co-repressor 2, and CD59. Top genes that are over expressed in the cisplatin sensitive cell line IGROV1 include thioredoxin interacting protein, insulin-like growth factor binding protein 1, pentatricopeptide repeat domain 3 and chloride intracellular channel 4. Additionally, we identified many novel genes without functional annotations, such as hypothetical protein MGC61571 and chromosome 17 open reading frame 81, as among the top differentially expressed genes.

We applied GO term enrichment analysis to the over or under expressed genes using GO miner with level 3 categories. The enriched GO terms with log10(P) > 2 are shown in Figure 1–2 and in Supplementary Table 2. We found that GO:0001837 (epithelial to mesenchymal transition) is enriched in genes that are over expressed in IGROV1-CP cells with 4 out of 9 genes (Table 2) in this GO term changed significantly in expression levels. These four genes are: CTNNB1 (catenin beta 1), S100A4 (S100 calcium binding protein A4), TGFB1 (transforming growth factor, beta 1) and BMP7 (bone morphogenetic protein 7). Their expression levels in IGROV1-CP and IGROV1 are shown in Table 2.

Another interesting GO term enriched in genes that are over expressed in IGROV1-CP cells is the GO term GO:0034330 (cell junction assembly and maintenance) with six genes over expressed in IGROV1-CP cells compared to IGROV1 cells (Table 2).

For genes over expressed in IGROV1, the cisplatin sensitive cell line, we found that multiple enriched GO terms related to cell death are enriched (Figure 2 and Supplementary Table 2), which include GO:0008219 cell death, GO:0012501 programmed cell death, GO:0043067 regulation of programmed cell death and GO:0006915 apoptosis. For example, 61 genes of 426 genes related to GO:0006915 apoptosis is over expressed in IGROV1 cells compared to IGROV1-CP cells (data not shown).

SBS analysis of chemotherapy resistant and sensitive tissues of ovarian cancer

In order to study the chemotherapy response program in clinical samples, we selected two ovarian cancer tissues: one with complete response and the other with a persistent resistance to chemotherapy. The clinical information of the two cases is shown in Table 3. It is not easy to obtain these extreme cases as most of the tissues show partial response to chemotherapy. After SBS sequencing, 1,762,449 and 1,290,935 tags could be mapped to the human genome for the ovarian cancer tissue sample; 12,344 and 12,628 respectively. These tags can be reduced to 20,775 unique genes.

Unlike MPSS analysis, in which multiple sequencing runs (two-stepper or four steppers) were generated [4,11], SBS does not generate replicate runs. The Z-test that we used in the MPSS analysis was not applicable here for the analysis of the SBS data. We have therefore applied another program named intensity-dependent selection of expression ratios (ISER)[13] (https://ipe.cbmeg.unicamp.br/pub/PMmA), which was developed to analyze DNA array data when little or no replication of array hybridizations is available. Using a P value < 0.05, we identified a total of 4,348 differentially expressed genes. As differential expression of those low expression genes might not be reliably detected, we additionally set the filter so that the sum of the expression would be at least 30 TPM for the two samples. In the end, we identified 1,187 tags (corresponding to 1,040 genes) that were differentially expressed between the chemotherapy responsive tissue and the chemotherapy persistent resistant tissue (Supplementary Table 3).

Applying Gene Ontology analysis, we found that GO:0050673 epithelial cell proliferation and GO:0050678 regulation of epithelial cell proliferation were enriched in the genes over expressed in the chemotherapy resistant tissue while the GO:0007229 integrin-mediated signaling pathway was enriched in the genes over expressed in the chemotherapy sensitive tissue (Figure 3 and Supplementary Table 4). The genes involved in these GO terms and their associated expression values are shown in Table 4. Three gene COL3A1, PTK2 and ADAMTS1, which belong to the GO:0007229 integrin-mediated signaling pathway, were over expressed in the chemotherapy sensitive tissue when compared to the chemotherapy resistant tissue. In addition, three laminin genes (LAMA1, LAMB1, LAMC1), keratin 4 and cyclin-dependent kinase inhibitor 1C (p57, Kip2) were over expressed in the chemotherapy resistant tissue when compared to the chemotherapy sensitive tissue (Table 4).

Integrative analysis of ovarian cancer cell line cisplatin resistant model and the ovarian cancer tissue resistant model

The cell line model of IGROV1-CP and IGROV1 represents the acquired resistant model for cisplatin, as IGROV-1-CP cells are cisplatin resistant ovarian cancer cells derived from its cisplatin sensitive parental IGROV-1 cells after prolonged exposure to cisplatin compound and selection of resistant clones [7]. However, carboplatin and paclitaxel combination treatment is the standard first-line chemotherapy regimen [8,9]. The analysis using the chemotherapy resistant and sensitive ovarian cancer tissues thus represents a model for the combination chemotherapy response. We were curious whether there are common mechanisms or common differentially regulated genes between these two models. Using gene name as common identifiers, we identified a total of 228 genes (21.9%, 228/1,040 genes) that are common to the two analyses (Supplementary Table 5). Among them, 110 genes changed in the same direction in the comparison between chemotherapy resistant (IGROV1-CP or the chemoresistant tissue 12344) vs. chemotherapy sensitive (IGROV1 or the chemosensitive tissue 12628) samples (Table 5). This list includes many interesting genes such as two BMP proteins (BMP4 and BMP7), six solute carrier proteins, transcription factor POU5F1 (POU class 5 homeobox 1), and KLK10 (kallikrein-related peptidase 10).

Gene Ontology analysis of this 110 common list of genes revealed many interesting GO terms that are enriched including GO:0050679 positive regulation of epithelial cell proliferation and GO:0046942 carboxylic acid transport (Table 6). Three genes belonging to GO:0046942 carboxylic acid transport are SLC25A1, SLC16A3 and SLC7A5.

Recently, the Cancer Genome Atlas (TCGA) recently deposited and released some next-generation sequencing data for ovarian cancers on the TCGA data portal. Sixteen of the samples were defined as complete response samples. However, only three samples were defined as progressive disease samples. Our term "chemosensitive" is comparable to the term "complete response" used in TCGA, and "chemoresistant" is comparable to the term "progressive disease" used in TCGA. We downloaded the raw data file, processed to get differentially expressed genes between the complete response group and the progressive disease group. We then compared the differentially expressed genes (using 1.5 fold as cutoff values) of the TCGA data and the differentially expressed genes we identified in table 5. We were able to found 15 genes that were down regulated in the complete response group compared with the progressive disease group. Among them, 11 genes (73%) also showed as down regulated in the chemosensitive group compared with the chemoresistant group in our sequencing data. In addition, among the 6 genes that were identified as over expressed in the TCGA data in the "progressive disease" group compared with the "complete response" group, 50% (3 of the 6) were consistent with our data (Supplementary Table 6). Considering the expected high degree of tumor heterogeneity and that the experiments were performed in different laboratories, an overall consistency of 67% (14/21) for both up- and down-regulated genes is pretty high, suggesting that the expression profiling by the next-generation sequencing used both by TCGA and us generated comparable data.

Network analysis of the commonly differentially expressed genes of ovarian cancer cell line cisplatin resistant model and the ovarian cancer tissue resistant model

In an attempt to gain further insight of the potential mechanism of the chemotherapy response based on the commonly differentially expressed genes of ovarian cancer cell line cisplatin resistant model and the ovarian cancer tissue resistant model, we took an approach to building a protein-protein interaction network of these proteins and then to identifying the proteins with the most interacting partners (i.e. network hubs) as key nodal control points for therapeutic intervention. We therefore analyzed the common gene list using Cytoscape with the MiMI plugin [16] to identify protein-protein interactions allowing a maximum of one neighbor to connect the proteins in the list. Cytoscape is a widely used open source software tool for displaying interactions of molecules [17]. The MiMi plugin for Cytoscape (http://mimiplugin.ncibi.org/) was developed to facilitate access to the molecular interaction data assembled in MiMI that contains integrated data from multiple well-known protein interaction databases using an intelligent deep-merging approach [18]. The MiMi plugin retrieves molecular interactions and interaction attributes from MiMI and displays the interaction networks and attributes using Cytoscape [16]. In the end, we obtained a network contains 540 nodes and 1907 edges (Supplementary Figure 1). The sif file for the combined network is shown in Supplementary Table 7.

As the resulting network is complicated, we tried to identify key nodal points in the network using MCODE [19], a Cytoscape plugin that finds clusters (highly interconnected regions) in a network. We identified an over expressed subnetwork that is over expressed in the chemotherapy resistant tissue or in the cisplatin resistant IGROV1-CP cells compared to the chemotherapy sensitive tissue or cisplatin sensitive IRGOV1 cells. This subnetwork contains three genes that are over expressed in the chemoresistant tissue or cells. They are: BMP7 (bone morphogenetic protein 7), NR2F2 (nuclear receptor subfamily 2, group F, member 2) and AP2B1 (Adaptor-related protein complex 2, beta 1 subunit) (Figure 4). However, we did not

find any significant network cluster with genes over expressed in the chemosensitive tissue or cells using the MCODE program.

Discussion

We applied two next-generation sequencing technologies (the MPSS and the SBS technology) to study two ovarian chemotherapy response models: the acquired cisplatin resistant cell line model (IGROV1-CP and IGROV1) and the ovarian cancer tissue resistant model. Our database offers the first comprehensive view of the digital transcriptomes of ovarian cancer cell lines and tissues with different chemotherapy response phenotypes. These are the first steps toward a systems approach to disease through an integrative, systemic understanding of chemotherapy response at system and network levels. We previously reported a limited proteomics analysis of 1117 proteins in IGROV1 and IGROV1-CP cells, with integrated analysis of these proteins with their corresponding transcript levels obtained by the MPSS technology [20]. In this manuscript, we reported the complete data set for the MPSS analysis, and the integrated analysis of the MPSS data with the SBS data, which was newly generated for the chemotherapy resistant and sensitive ovarian cancer tissues.

From the MPSS analysis, we observed that many genes showed a pattern of high expression in one cellular state (e.g. the cisplatin resistant state of IGROV1-CP) but no expression in another state (Table 1 and Supplementary Table 1). Many interesting genes are among the top genes that showed such expression patterns (Table 1). For example, the MIF (macrophage migration inhibitory factor) gene is expressed at 984 TPM in the cisplatin resistant IGROV1-CP cells, but shows no expression in the cisplatin sensitive IGROV1 cells. Agarwal et al. showed that ovarian cancer cells, but not normal cells, secrete significant amounts of MIF and that serum MIF levels are significantly higher in ovarian cancer patients compared with normal individuals [6]. Hagemann et al. showed that ovarian cancer cell-derived migration inhibitory factor enhances tumor growth, progression, and angiogenesis [21]. Krockenberger et al. found that MIF may contribute to the immune escape of ovarian carcinoma [22]. In contrast, IGFBP1 (insulin-like growth factor binding protein 1) is a gene that is highly expressed in the cisplatin sensitive IGROV1 cells (473 TPM) but shows no expression in the cisplatin resistant IGROV1-CP cells. However, the association of IGFBP1 and ovarian cancer chemotherapy has not been reported and its underlying mechanism is not known.

Gene ontology analysis revealed that GO:0001837 (epithelial to mesenchymal transition) is enriched in genes that are over expressed in IGROV1-CP cells compared to IGROV1 cells, suggesting that the EMT process may be related to the acquired resistance to chemotherapy. Four of the genes in this GO terms were over expressed in IGROV1-CP cells compared to IGROV1 cells, which include CTNNB1 (catenin beta 1), S100A4 (S100 calcium binding protein A4), TGFB1 (transforming growth factor, beta 1) and BMP7 (bone morphogenetic protein 7) (Table 2). Epithelial-to-mesenchymal transition (EMT) is a vital process for morphogenesis during embryonic development. It is also implicated in the conversion of early stage tumors into invasive malignancies by molecular changes that facilitates cell motility and invasion [23]. Yang et al. recently showed that the oxaliplatin-resistant (OxR) colorectal cancer (CRC) cells underwent EMT characterized by the phenotypic changes including spindle-cell shape, loss of polarity, intercellular separation, and pseudopodia formation [24]. Oxaliplatin is another platinum-based cancer chemotherapy drug similar to cisplatin [25]. Our data suggest that the EMT process may also play a role in the transition of cisplatin sensitive IGROV1 to cisplatin resistant IGROV1-CP cells.

There may exist different mechanisms for conferring chemotherapy resistance in the cell line model vs. the tissue model. In the cell line model, the sensitivity to cisplatin seems to be related to increased cell deaths and apoptosis (Supplementary Table 2). However, in the tissue model,

other extracellular factors may also play roles. Tissues are a mixture of epithelial and stromal cells, and the stromal components including extracelluar matrix proteins may play roles in modulating drug penetrance, and thus modifying chemotherapy responses. We found that in the tissue model, genes involved in the extracellular matrix such as laminin genes (e.g. LAMA1, LAMB1, LAMC1), keratins (e.g. keratin 4), and collagens (e.g. COL3A1) were differentially expressed. This observation is similar to the findings in our recent quantitative proteomics analysis of proteins differentially expressed between chemotherapy sensitive tissues and chemotherapy resistant tissues, where we identified multiple proteins belonging to the extracellular matrix (ECM) that are over expressed in the chemotherapy resistant tissues [26]. These proteins include decorin, versican, basigin (CD147), fibulin-1, extracellular matrix protein 1, biglycan, fibronectin 1, dermatopontin, alpha-cardiac actin (smooth muscle actin), and an EGF-containing fibulin-like extracellular matrix protein 1 [26].

An integrative analysis of the cell line and tissue model of ovarian cancer chemotherapy responses revealed many common genes that may be the key players in conferring chemotherapy resistance (Table 5). For example, the six solute carrier proteins SLC10A3, SLC16A3, SLC25A1, SLC35B3, SLC7A5, and SLC7A7 belong to the common list, suggesting that these solute carrier proteins may modulate the influx/efflux of drugs from cells and thus modulate chemotherapy response. They may be key therapeutic targets for improving chemotherapy response in ovarian cancers.

A network analysis revealed a subnetwork with three gene BMP7, NR2F2 and AP2B1 that were consistently over expressed in the chemoresistant tissue or cells (Figure 4) compared to the chemosensitive tissue of cells. Our finding is novel because, to our knowledge, the association of these three genes with chemotherapy has not been reported before, nor did the interaction among these genes. BMP4 is a member of the TGF beta family and is involved in TGF beta signaling pathway [27]. Recently, Theriault et al. showed that BMP4 treatment of ovarian cancer cells increased their cellular adhesion, motility and invasiveness [28]. NR2F2, also named chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), is a nuclear steroid receptor [29] and was initially identified as a transcription factor for the chicken ovalbumin gene [30]. Navab et al. showed that expression of NR2F2 enhanced the invasiveness of human lung carcinoma cells [31]. AP2B1 is one of two large chain components of the assembly protein complex 2, which serves to link clathrin to receptors in coated vesicles [32]. Not much is known about AP2B1 and cancer. We showed here that over expression of these three genes in the subnetwork was associated with resistance to chemotherapy of ovarian cancer cells. The mechanism of over expression of BMP7, NR2F2 and AP2B1 in this subnetwork in conferring chemotherapy resistance warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

MPSS   massively parallel signature sequencing

| SBS | sequencing by synthesis |
| TPM | transcripts per million |

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LOG10(p)	Go terms enriched in genes that are over expressed in IGROV1-CP cells
	GO CATEGORY
-6.97	GO:0044237_cellular_metabolic_process
-6.91	GO:0008152_metabolic_process
-6.78	GO:0006412_translation
-6.30	GO:0044238_primary_metabolic_process
-6.29	GO:0043170_macromolecule_metabolic_process
-5.79	GO:0044249_cellular_biosynthetic_process
-5.22	GO:0019538_protein_metabolic_process
-4.69	GO:0044267_cellular_protein_metabolic_process
-4.23	GO:0044260_cellular_macromolecule_metabolic_process
-4.04	GO:0010467_gene_expression
-3.73	GO:0009987_cellular_process
-3.63	GO:0009058_biosynthetic_process
-3.50	GO:0009059_macromolecule_biosynthetic_process
-3.50	GO:0016043_cellular_component_organization_and_biogenesis
-3.45	GO:0006396_RNA_processing
-3.37	GO:0006807_nitrogen_compound_metabolic_process
-3.18	GO:0016192_vesicle-mediated_transport
-3.18	GO:0022607_cellular_component_assembly
-2.96	GO:0001837_epithelial_to_mesenchymal_transition
-2.81	GO:0006519_amino_acid_and_derivative_metabolic_process
-2.52	GO:0006575_amino_acid_derivative_metabolic_process
-2.52	GO:0065003_macromolecular_complex_assembly
-2.49	GO:0051052_regulation_of_DNA_metabolic_process
-2.40	GO:0008380_RNA_splicing
-2.38	GO:0034330_cell_junction_assembly_and_maintenance
-2.35	GO:0006457_protein_folding
-2.34	GO:0009308_amine_metabolic_process
-2.27	GO:0014031_mesenchymal_cell_development
-2.27	GO:0048762_mesenchymal_cell_differentiation
-2.22	GO:0016202_regulation_of_striated_muscle_development
-2.22	GO:0048634_regulation_of_muscle_development
-2.10	GO:0019320_hexose_catabolic_process
-2.10	GO:0046164_alcohol_catabolic_process
-2.10	GO:0046365_monosaccharide_catabolic_process
-2.02	GO:0044275_cellular_carbohydrate_catabolic_process
-2.01	GO:0051171_regulation_of_nitrogen_compound_metabolic_process
-2.01	GO:0000018_regulation_of_DNA_recombination

Figure 1.
A chart showing the over represented Gene Ontology terms in genes over expressed in IGROV1-CP cells comparing to IGROV1 cells. The shade of the red color indicates the P values of enrichments [Log10(P)].

	Go terms enriched in genes that are under expressed in IGROV1-CP cells
-10.42	GO:0044237_cellular_metabolic_process
-9.57	GO:0044238_primary_metabolic_process
-9.54	GO:0008152_metabolic_process
-7.93	GO:0010467_gene_expression
-7.65	GO:0006139_nucleobase__nucleoside__nucleotide_and_nucleic_acid_metabolic_process
-7.27	GO:0043170_macromolecule_metabolic_process
-6.33	GO:0043283_biopolymer_metabolic_process
-5.56	GO:0022613_ribonucleoprotein_complex_biogenesis_and_assembly
-5.38	GO:0016070_RNA_metabolic_process
-5.30	GO:0016071_mRNA_metabolic_process
-5.11	GO:0022618_protein-RNA_complex_assembly
-4.87	GO:0000377_RNA_splicing__via_transesterification_reactions_with_bulged_adenosine_as_nucleophile
-4.87	GO:0000398_nuclear_mRNA_splicing__via_spliceosome
-4.71	GO:0000375_RNA_splicing__via_transesterification_reactions
-4.60	GO:0006397_mRNA_processing
-4.34	GO:0006412_translation
-4.07	GO:0006396_RNA_processing
-3.84	GO:0008380_RNA_splicing
-3.83	GO:0009059_macromolecule_biosynthetic_process
-3.83	GO:0009058_biosynthetic_process
-3.62	GO:0044249_cellular_biosynthetic_process
-3.31	GO:0065003_macromolecular_complex_assembly
-3.04	GO:0008219_cell_death
-3.04	GO:0016265_death
-2.98	GO:0044267_cellular_protein_metabolic_process
-2.98	GO:0009987_cellular_process
-2.97	GO:0022607_cellular_component_assembly
-2.89	GO:0006413_translational_initiation
-2.85	GO:0046907_intracellular_transport
-2.81	GO:0043285_biopolymer_catabolic_process
-2.76	GO:0050794_regulation_of_cellular_process
-2.76	GO:0006350_transcription
-2.66	GO:0012501_programmed_cell_death
-2.66	GO:0009056_catabolic_process
-2.62	GO:0019538_protein_metabolic_process
-2.62	GO:0043067_regulation_of_programmed_cell_death
-2.59	GO:0019222_regulation_of_metabolic_process
-2.55	GO:0007005_mitochondrion_organization_and_biogenesis
-2.52	GO:0031323_regulation_of_cellular_metabolic_process
-2.50	GO:0006915_apoptosis
-2.50	GO:0044248_cellular_catabolic_process
-2.45	GO:0042981_regulation_of_apoptosis
-2.44	GO:0009057_macromolecule_catabolic_process
-2.39	GO:0044260_cellular_macromolecule_metabolic_process
-2.37	GO:0006417_regulation_of_translation
-2.33	GO:0006376_mRNA_splice_site_selection
-2.32	GO:0010468_regulation_of_gene_expression
-2.31	GO:0050789_regulation_of_biological_process
-2.25	GO:0006886_intracellular_protein_transport
-2.17	GO:0043281_regulation_of_caspase_activity
-2.17	GO:0006919_caspase_activation
-2.16	GO:0043624_cellular_protein_complex_disassembly
-2.15	GO:0048468_cell_development
-2.14	GO:0051246_regulation_of_protein_metabolic_process
-2.11	GO:0006605_protein_targeting
-2.10	GO:0052547_regulation_of_peptidase_activity
-2.10	GO:0052548_regulation_of_endopeptidase_activity
-2.08	GO:0051641_cellular_localization
-2.05	GO:0000389_nuclear_mRNA_3'-splice_site_recognition
-2.05	GO:0015908_fatty_acid_transport
-2.05	GO:0043244_regulation_of_protein_complex_disassembly
-2.05	GO:0048259_regulation_of_receptor-mediated_endocytosis
-2.03	GO:0051128_regulation_of_cellular_component_organization_and_biogenesis
-2.02	GO:0015031_protein_transport
-2.01	GO:0006366_transcription_from_RNA_polymerase_II_promoter
-2.01	GO:0043241_protein_complex_disassembly
-1.96	GO:0051649_establishment_of_cellular_localization

Figure 2.
A chart showing the over represented Gene Ontology terms in genes over expressed in IGROV1 cells comparing to IGROV1-CP cells. The shade of the blue-cyan color indicates the P values of enrichments [Log10(P)].

LOG10(p)	Enriched in Chemo-R over expressed genes
-3.42	GO:0009888_tissue_development
-3.11	GO:0050789_regulation_of_biological_process
-3.06	GO:0050673_epithelial_cell_proliferation
-3.06	GO:0050678_regulation_of_epithelial_cell_proliferation
-2.92	GO:0050794_regulation_of_cellular_process
-2.92	GO:0007398_ectoderm_development
-2.85	GO:0010468_regulation_of_gene_expression
-2.85	GO:0043086_negative_regulation_of_catalytic_activity
-2.77	GO:0048519_negative_regulation_of_biological_process
-2.71	GO:0008544_epidermis_development
-2.61	GO:0048523_negative_regulation_of_cellular_process
-2.51	GO:0065007_biological_regulation
-2.33	GO:0009892_negative_regulation_of_metabolic_process
-2.23	GO:0050679_positive_regulation_of_epithelial_cell_proliferation
-2.12	GO:0032502_developmental_process
-2.10	GO:0031324_negative_regulation_of_cellular_metabolic_process
-2.08	GO:0045787_positive_regulation_of_cell_cycle
-2.06	GO:0006469_negative_regulation_of_protein_kinase_activity
-2.06	GO:0033673_negative_regulation_of_kinase_activity
-2.00	GO:0045449_regulation_of_transcription
-2.00	GO:0051348_negative_regulation_of_transferase_activity
	Enriched in Chemo-S over expressed genes
-2.80	GO:0007229_integrin-mediated_signaling_pathway
-2.40	GO:0048856_anatomical_structure_development
-2.23	GO:0001516_prostaglandin_biosynthetic_process
-2.23	GO:0008105_asymmetric_protein_localization
-2.23	GO:0046457_prostanoid_biosynthetic_process
-2.16	GO:0032502_developmental_process
-2.06	GO:0008544_epidermis_development
-1.96	GO:0005976_polysaccharide_metabolic_process

Figure 3.
A chart showing the over represented Gene Ontology terms in genes over expressed in chemosensitive (Chemo-S) or chemoresistant (Chemo-R) tissues. The shade of the blue or red colors indicates the P values of enrichments [Log10(P)].
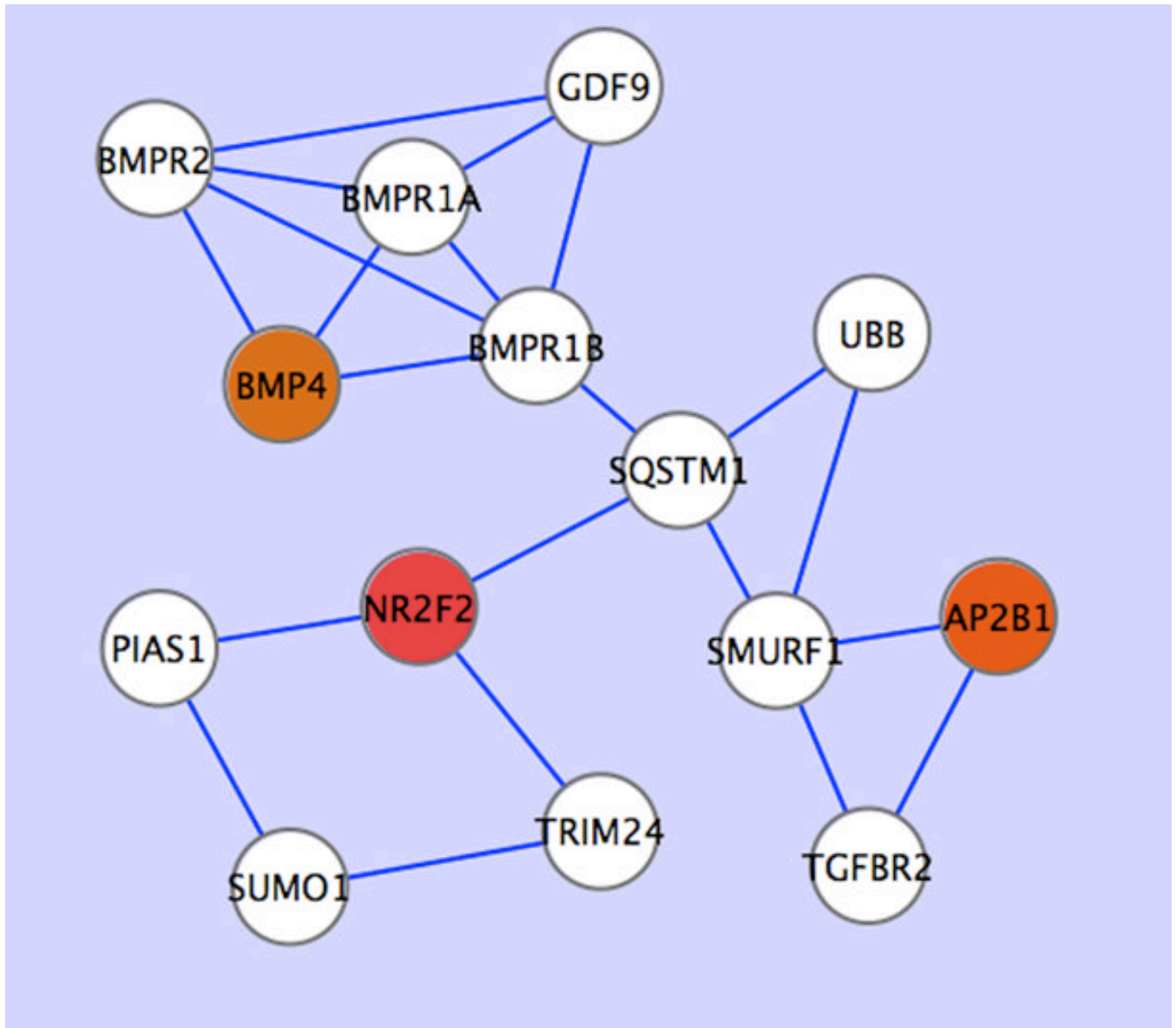
Figure 4.
A subnetwork that is over expressed in the chemoresistant cells compared to the chemosensitive cells. The log2 expression ratios of the chemoresistant tissue to the chemosensitive tissue were overlaid onto the network. Red color indicates over expression and white color indicates no significant differentially expression. Circles indicate nodes (proteins) and lines indicate protein-protein interaction taken from the Human Protein Reference Database (HPRD) (http://www.hprd.org/).

Table 1

Top 20 up and down regulated genes in IGROV1_CP comparing to IGROV1

Gene Name	IGROV1_CP (tpm)	IGROV1 (tpm)	Ratio*	Description	GO biological functions
NEK8	0	106	0	NIMA (never in mitosis gene a)- related kinase 8	protein amino acid phosphorylation
PARVB	0	88	0	Parvin, beta	cell adhesion
CLIC4	0	88	0	Chloride intracellular channel 4	chloride transport, negative regulation of cell migration
EIF3EIP	0	95	0	Eukaryotic translation initiation factor 3, subunit E interacting protein	initiation of protein synthesis
FHIT	0	103	0	Fragile histidine triad gene	DNA replication, diadenosine triphosphate catabolic process
AGPAT1	135	0	infinite	1-acylglycerol-3- phosphate O- acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	positive regulation of cytokine production, phospholipid metabolic process
MGC61571	0	99	0	Hypothetical protein MGC61571	unknown
MRPL1	0	175	0	Mitochondrial ribosomal protein L1	RNA processing, translation
EIF2AK1	0	164	0	Eukaryotic translation initiation factor 2-alpha kinase 1	translation, cell differentiation, protein amino acid autophosphorylation
IGFBP1	0	473	0	Insulin-like growth factor binding protein 1	regulation of cell growth, signal transduction, insulin receptor signaling pathway
ANKMY2	134	0	infinite	Ankyrin repeat and MYND domain containing 2	unknown
GLT25D1	113	0	infinite	Glycosyltransferase 25domain containing 1	lipopolysaccharide biosynthetic process
ADCY1	0	145	0	Adenylate cyclase 1 (brain)	cAMP biosynthetic process, intracellular signaling cascade, hormone-mediated signaling

Table 2

Genes in GO term 0001837 and 0034330

GO terms	Gene Name	Description	IGROV1-CP[*]	IGROV1
GO:0001837_epithelial_to_mesenchymal_transition	CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	218	71
GO:0001837_epithelial_to_mesenchymal_transition	S100A4	S100 calcium binding protein A4	458	191
GO:0001837_epithelial_to_mesenchymal_transition	TGFB1	Transforming growth factor, beta 1	16	0
GO:0001837_epithelial_to_mesenchymal_transition	BMP7	Bone morphogenetic protein 7 (osteogenic protein 1)	180	0
GO:0034330_cell_junction_assembly_and_maintenance	LAMC1	Laminin, gamma 1 (formerly LAMB2)	821	408
GO:0034330_cell_junction_assembly_and_maintenance	PARD6A	Par-6 partitioning defective 6 homolog alpha (C. elegans)	19	0
GO:0034330_cell_junction_assembly_and_maintenance	TLN1	Talin 1	149	35
GO:0034330_cell_junction_assembly_and_maintenance	VCL	Vinculin	80	11
GO:0034330_cell_junction_assembly_and_maintenance	PRKCI	Protein kinase C, iota	26	0
GO:0034330_cell_junction_assembly_and_maintenance	ITGA6	Integrin, alpha 6	37	8

[*] expression in tpm

Table 3

Clincinal information of the two ovarian cancer tissues used for

Sample ID	Age	Stage	Grade	HistoType	Clinical Response	Recurrence
12344	62	IIIC	Moderately differentiated	serous	Persistent Resistance	Yes
12628	62	IIIC	Moderately differentiated	serous	Complete Response	No

Table 4

Genes in interesting enriched GO terms for differentially expressed genes between chemotherapy resistant and sensitive ovarian cancer tissues

GO term	Gene Name	Description	Sample_1234 4 (Chemo-R)	Sample__1262 8 (chemo-S)
GO:0007229_integrin- mediated_signaling_pathway	COL3A1	Collagen, type III, alpha 1 (Ehlers- Danlos syndrome type IV, autosomal dominant)	2242	7266
GO:0007229_integrin- mediated_signaling_pathway	PTK2	PTK2 protein tyrosine kinase 2	24	84
GO:0007229_integrin- mediated_signaling_pathway	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	74	473
GO:0050673_epithelial_cell_proliferation	LAMC1	Laminin, gamma 1 (formerly LAMB2)	201	50
GO:0050673_epithelial_cell_proliferation	LAMA1	Laminin, alpha 1	66	8
GO:0050673_epithelial_cell_proliferation	KRT4	Keratin 4	101	11
GO:0050673_epithelial_cell_proliferation	CDKN1C	Cyclin- dependent kinase inhibitor 1C (p57, Kip2)	144	15
GO:0050673_epithelial_cell_proliferation	LAMB1	Laminin, beta 1	78	18

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Table 5

Genes that are changed in the same direction in the cell line model and the ovarian cancer tissue model

common genes	Description	IGROV1_CP	IGROV1	12344 (Chemo- R)	12628 (chemo- S)
CYP26B1	Cytochrome P450, family 26, subfamily B, polypeptide 1	46	0	67	2
AP2B1	Adaptor-related protein complex 2, beta 1 subunit	52	8	110	14
PSMB1	Proteasome (prosome, macropain) subunit, beta type, 1	151	70	976	265
LYPLA2	Lysophospholipase II	182	96	199	24
MRC2	Mannose receptor, C type 2	64	154	101	375
FHL1	Four and a half LIM domains 1	36	2	489	46
RPL26L1	Ribosomal protein L26-like 1	20	0	160	46
NEDD4L	Neural precursor cell expressed, developmentally down-regulated 4- like	44	8	48	8
SP100	SP100 nuclear antigen	23	0	49	12
DUSP1	Dual specificity phosphatase 1	168	53	578	93
PABPC1	Poly(A) binding protein, cytoplasmic 1	5033	7569	1232	3655
SDHA	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	137	51	40	6
ADK	Adenosine kinase	55	0	61	17
ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1	98	23	139	35
TP53INP2	Tumor protein p53 inducible nuclear protein 2	37	0	61	14
LAMB1	Laminin, beta 1	726	234	78	18
PHGDH	Phosphoglycerate dehydrogenase	42	0	219	54
SLC25A1	Solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	103	293	25	100

Table 6

Enriched GO terms in the common differentially expressed genes between the cell line and the tissue model of ovarian cancer chemotherapy

GO CATEGORY	TOTAL GENES	CHANGED GENES	ENRICHMENT	LOG10(p)
GO:0050679_positive_regulation_of_epithelial_cell_proliferation	10	2	25.66	−2.59
GO:0032268_regulation_of_cellular_protein_metabolic_process	138	5	4.65	−2.38
GO:0046942_carboxylic_acid_transport	44	3	8.75	−2.33
GO:0015849_organic_acid_transport	45	3	8.55	−2.30
GO:0051246_regulation_of_protein_metabolic_process	148	5	4.33	−2.25
GO:0031323_regulation_of_cellular_metabolic_process	796	13	2.10	−2.19
GO:0016477_cell_migration	98	4	5.24	−2.16
GO:0019222_regulation_of_metabolic_process	810	13	2.06	−2.13
GO:0007005_mitochondrion_organization_and_biogenesis	55	3	7.00	−2.06
GO:0042325_regulation_of_phosphorylation	58	3	6.64	−1.99
GO:0045333_cellular_respiration	20	2	12.83	−1.99
GO:0050673_epithelial_cell_proliferation	20	2	12.83	−1.99
GO:0050678_regulation_of_epithelial_cell_proliferation	20	2	12.83	−1.99
GO:0007049_cell_cycle	317	7	2.83	−1.97