Differences in gene expression profiles and carcinogenesis pathways involved in cisplatin resistance of four types of cancer

YONG YANG[1,2], HUI LI[1,2], SHENGCAI HOU[1,2], BIN HU[1,2], JIE LIU[1,3] and JUN WANG[1,3]

[1]Beijing Key Laboratory of Respiratory and Pulmonary Circulation, Capital Medical University, Beijing 100069;
[2]Department of Thoracic Surgery, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020;
[3]Department of Physiology, Capital Medical University, Beijing 100069, P.R. China

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Abstract. Cisplatin-based chemotherapy is the standard therapy used for the treatment of several types of cancer. However, its efficacy is largely limited by the acquired drug resistance. To date, little is known about the RNA expression changes in cisplatin-resistant cancers. Identification of the RNAs related to cisplatin resistance may provide specific insight into cancer therapy. In the present study, expression profiling of 7 cancer cell lines was performed using oligonucleotide microarray analysis data obtained from the GEO database. Bioinformatic analyses such as the Gene Ontology (GO) and KEGG pathway were used to identify genes and pathways specifically associated with cisplatin resistance. A signal transduction network was established to identify the core genes in regulating cancer cell cisplatin resistance. A number of genes were differentially expressed in 7 groups of cancer cell lines. They mainly participated in 85 GO terms and 11 pathways in common. All differential gene interactions in the Signal-Net were analyzed. CTNNB1, PLCG2 and SRC were the most significantly altered. With the use of bioinformatics, large amounts of data in microarrays were retrieved and analyzed by means of thorough experimental planning, scientific statistical analysis and collection of complete data on cancer cell cisplatin resistance. In the present study, a novel differential gene expression pattern was constructed and further study will provide new targets for the diagnosis and mechanisms of cancer cisplatin resistance.

Correspondence to: Professor Hui Li, Department of Thoracic Surgery, Beijing Chao-Yang Hospital, Capital Medical University, 8 Workers Stadium South Road, Beijing 100020, P.R. China
E-mail: huilee@vip.sina.com

Professor Jun Wang, Beijing Key Laboratory of Respiratory and Pulmonary Circulation, Capital Medical University, Beijing 100069, P.R. China
E-mail: wang_jun@ccmu.edu.cn

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Introduction

Cisplatin is primarily effective through DNA damage and is widely used for the treatment of several types of cancer, such as testicular, lung and ovarian cancer. However, the ability of cancer cells to become resistant to cisplatin remains a significant impediment to successful chemotherapy. Although previous studies have identified numerous mechanisms in cisplatin resistance, it remains a major problem that severely limits the usefulness of this chemotherapeutic agent. Therefore, it is crucial to examine more elaborate mechanisms of cisplatin resistance in order to find new targets to prevent drug resistance. Following the rapid development of molecular biology technology, it is possible to detect the molecular differences between the different cells which may provide us with important insights into drug resistance. Thus, it is critical to understand the relationships between cisplatin resistance and molecular changes, as this may aid in the diagnosis of cisplatin resistance and in the improvement of the therapeutic effects of cisplatin.

A number of studies have provided evidence for the molecular changes between cisplatin resistance and wild-type cell lines, indicating the abnormal expression of several genes including ERCC1 and MRP1. However, limited by the development of techniques, several previous studies on cisplatin resistance had difficulties in assisting with clinical research. Firstly, most studies investigated a single molecule, while which pathways it applied to remains unclear. Secondly, most investigated mechanisms referred to a single cancer cell line, and whether a certain hypothesis may apply to another cancer cell line in cisplatin resistance remains unknown.

The advent of genome-wide technologies, such as gene expression microarray, has made it possible to achieve a comprehensive view of the alteration involved in drug resistance. Although several results have been published on cancer cisplatin resistance and although their primary gene expression profile data have been uploaded to the GEO database, no studies have yet combined and investigated these data. In this study, 7 group of cell lines were investigated using gene microarray analysis to examine the differences in gene expression between the cisplatin resistant and wild-type cell lines including the non-small cell lung cancer (NSCLC) cell lines A549 and H460, the ovarian cancer cell line A2780, the oral squamous cancer cell line KB-3-1 and the testicular cancer cell lines 833K, GCT27 and Susa.

Table I. Characteristics of the 7 cancer cell lines used.

	Cell line	Characteristics	No. of microarrays
1	A549	Human non-small cell lung cancer cell line	3
2	H460	Human non-small cell lung cancer cell line	3
3	A2780	Human ovarian cancer cell line	6
4	KB-3-1	Human oral squamous cancer cell line	2
5	833K	Human TGCT cell line	2
6	GCT27	Human TGCT cell line	2
7	Susa	Human TGCT cell line	2

Materials and methods

Cell line selection. Cell line microarray data were obtained from 7 datasets including 2 NSCLC cell lines A549 and H460, 1 ovarian cancer cell line A2780, 1 oral squamous cancer cell line KB-3-1 and 3 testicular germ cell tumor (TGCT) cell lines 833K, GCT27 and Susa. The 7 datasets included 1 pair of cisplatin-resistant and 1 pair of wild-type cancer cell lines. Microarray analyses of NSCLC cell lines A549 and A549/CDDP were performed by us using Arraystar Human LncRNA Microarray V2.0 which contained 30,215 coding transcripts. The other 6 data series were accessible at NCBI GEO database, accession numbers were GSE 21656, GSE 33482, GSE 19397 and GSE 14231. The characteristics of the cancer cell lines are presented in Table I.

Differential gene expression. As some data series had only 2 replicates for arrays in each group, genes differentially expressed between normal and cisplatin-resistant cancer cell lines were identified using the t-test method. Using t-test and the tumors with wild-type as the control group, the P-value and the fold-change were calculated for each differential expression gene. With a threshold of P-value <0.05 and fold-change ≥1.5, cisplatin resistance-related differential expression genes were selected. Unsupervised hierarchical clustering was performed with cluster using Pearson's correlation distance metric and average linkage followed by visualization in Treeview (1).

Gene Ontology (GO) analysis. Based on the GO Database (http://www.geneontology.org/), the significance level of GOs of the cisplatin resistance-related differentially expressed genes was analyzed by the two-sided Fisher's exact test and using DAVID (http://david.abcc.ncifcrf.gov/home.jsp) analysis (2). The differential expression genes were analyzed independently according to up- and downregulation of these genes. We computed P-values for all the differential expression genes in all GO categories, and P-value <0.01 was considered to indicate statistically significant differences.

Pathway analysis. Based on the KEGG (http://www.genome.jp/kegg/) database, the significance level of pathways of the cisplatin resistant-related differentially expressed genes was analyzed by Pathway-Express (3,4). Significant differences from the expected were calculated with a two-sided binomial distribution. The number of genes corresponding to each pathway category among the differentially expressed genes was tallied and compared with the number of genes expected for each pathway category. Significant differences from the expected were calculated with a two-sided binomial distribution. All signaling pathways were analyzed for the significance level, using γ $P<0.05$ as the threshold.

Signal-Net analysis. Using java that allows users to build and analyze molecular networks, network maps were constructed. For instance, if there is confirmative evidence that 2 genes interact with each other, an interaction edge is assigned between the 2 genes. The considered evidence is the source of the interaction database from KEGG. Networks are stored and presented as graphs, where nodes are mainly genes (including protein, compound) and edges represent relation types between the nodes, such as activation or phosphorylation. The graph nature of networks led us to investigate them with powerful tools implemented in R.

To investigate the global network, we computationally identified the most important nodes. Thus, we turned to the connectivity (also known as degree) defined as the sum of connection strengths with the other network genes:

$$K_i = \sum_{u \neq i} a_{ui}$$

In gene networks, the connectivity measures how a gene correlates with all other network genes. For a gene in the network, the number of source genes of a gene is called the indegree of the gene and the number of target genes of a gene is its outdegree. The character of genes is described by betweenness centrality measures reflecting the importance of a node in a graph relative to other nodes. For a graph G: (V, E) with n vertices, the relative betweenness centrality $C_B(V)$ is defined by:

$$C_B(v) = \frac{2}{n^2 - 3n + 2} \sum_{\substack{s \neq v \neq t \in V \\ s \neq t}} \frac{\sigma_{st}(v)}{\sigma_{st}}$$

where σ_{st} is the number of shortest paths from s to t, and $\sigma_{st}(V)$ is the number of shortest paths from s to t that pass through a vertex v (5-9).

Data analysis. The numerical data are presented as the means ± standard deviation (SD). Differences between means were analyzed using the Student's t-test. All statistical analyses were performed using SPSS11.0 software (SPSS Inc., Chicago, IL, USA).

Results

Differential gene expression profiles in 7 pairs of cancer cell lines. Genome-wide transcriptional profiling of the tumor has demonstrated that extensive gene expression occurs following formation of cisplatin resistance. To investigate the possible gene expression change, a gene microarray study was used to analyze the mRNA expression profiles in 7 cell lines and their drug-resistant counterparts. Based on different types of gene chips, the number of differently expressed probes and total probes are listed in Table II. The list shows that each group of cell lines has a similar ratio of differential and total probes. In general, slightly more genes were upregulated than downregulated compared with the control cell lines. Hierarchical clustering showed systematic variations in the expression of mRNAs between the 2 cell lines (Fig. 1). The results demonstrated that these differential probes could clearly separate the 2 cell lines in all 7 groups.

GO analysis of differential genes in 7 pairs of cancer cell lines. Significant progress in data mining has provided a wide range of bioinformatics analysis options. For example, the GO, which has been proved to be highly beneficial for the mining of functional and biological significance from very large datasets (10,11), can produce a controlled vocabulary used for dynamic maintenance and interoperability between genome databases. GO analysis of differential genes in 7 pairs of cancer cell lines was performed by DAVID analysis. Seven groups of GO items merged together and 85 items that appeared >3 times in the 3 groups were obtained (Table III). In the biological part of the process, items regarding the downregulation of cell death appeared most times in the 7 cell line pairs and this corresponds to the drug resistance of the cells with high viability. By contrast, cell adhesion-related items generally presented more biological adhesion, cell adhesion and extracellular structure organization. In the cellular component, membrane-related items such as membrane fraction and cell-cell junction were upregulated compared with wild-type cell lines. In the molecular function subgroup, cytoskeletal protein binding and actin binding items variation emerged most in the group.

Pathway analysis of differential genes in 7 pairs of cancer cell lines. The oncogenetic pathways of cisplatin-resistant cancer cell lines were analyzed according to the functions and interactions of the differential genes. By using Pathway-Express which contains both the up- and downregulated differential genes in its analysis and the threshold of significance defined on the basis of γ P-value ≤ 0.05, tens of significant pathways were found (Figs. 2-8). To investigate the frequency in these 7 pairs of cell lines, repeating pathways that appeared in more than half (4 times) were collected and are listed in Table IV. In this table, 11 pathways are listed and phosphatidylinositol signaling system, cell adhesion molecules (CAMs), and leukocyte transendothelial migration appeared in all 7 groups. Furthermore, in the cancer pathway, TGF-β signaling pathway and focal junction have also been reported associated with cisplatin resistance. Therefore, pathway analysis showed us an equally important role and function as GO analysis.

Table II.

	Cell line	Gene chips	No. of upregulated probes	No. of downregulated probes	Total probes	Rate of upregulated probes	Rate of downregulated probes	Upregulated/ downregulated
1	A549	Arraystar Human LncRNA Microarray V2.0	1083	917	18844	0.057471874	0.048662704	1.181025082
2	H460	Affymetrix Human Gene 1.0 ST Array	282	280	33297	0.008469231	0.008409166	1.007142857
3	A2780	Agilent-014850 Whole Human Genome Microarray 4x44K G4112F	2480	2783	43376	0.057174474	0.064159904	0.891124686
4	KB-3-1	Affymetrix Human Genome U133A Array	344	303	22283	0.015437778	0.01359781	1.135313531
5	833K	Affymetrix Human Genome U133 Plus 2.0 Array	318	274	54675	0.005816187	0.005011431	1.160583942
6	GCT27	Affymetrix Human Genome U133 Plus 2.0 Array	524	455	54675	0.009583905	0.008321902	1.151648352
7	Susa	Affymetrix Human Genome U133 Plus 2.0 Array	883	840	54675	0.016149977	0.015363512	1.051190476

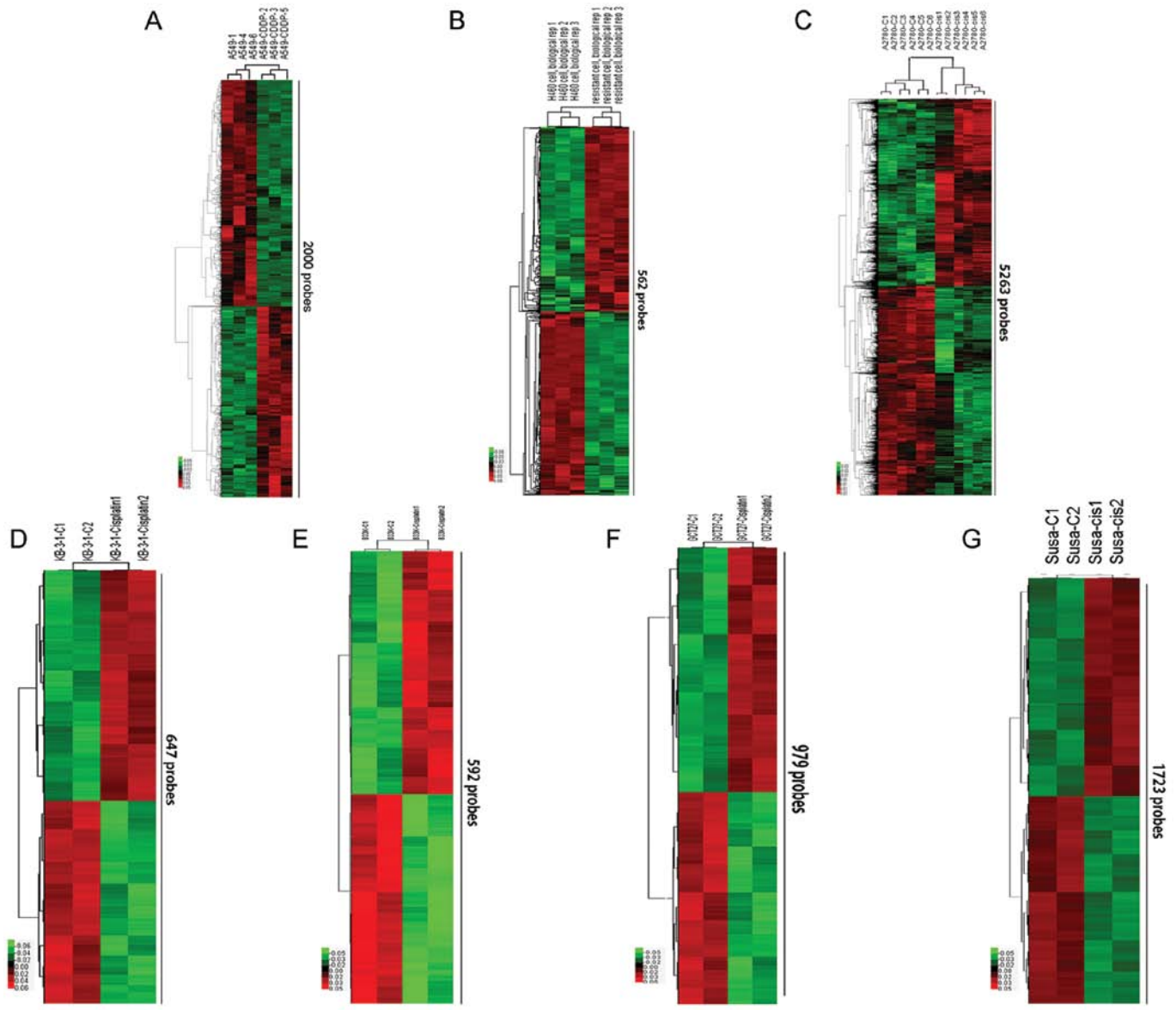

Figure 1. Unsupervised classification of cancer cell lines based on gene expression profiling. Classification of 7 pairs of cancer cell lines using the probe sets identified as differentially expressed between the cisplatin-resistant cell line and their own wild-type samples. Expression data are depicted as a data matrix where each row represents a probe and each column represents a sample. Expression levels are depicted according to the color scale shown at the top. Red and green indicate expression levels, respectively, above and below the median. The magnitude of deviation from the median is represented by the color saturation. (A-G) Represents the heat map of A549, H460, A2780, KB-3-1, 833K, GCT27 and Susa, respectively.

Signal transduction networks composed of 7 pairs of cancer cell lines. According to the literature and experimental records in the databases, 403 genes appearing in previous 11 pathways were collected and a diagram of the gene interaction network was drawn up based on these genes (Fig. 9). The total number of genes in the network was 337, and the specific relationships between them are listed in Table V. In the network, cycle nodes represent genes and edges between 2 nodes represent interactions between genes, which were quantified by degree. Degrees within the network which describe the number of single genes that regulate other genes represent the size of the cycle node. The higher the degree, the more central the gene occurs within the network. The clustering coefficient can be used to estimate the complexity of interactions among genes that neighbor the core gene with the exception of core gene participation. The lower the clustering coefficient, the more independent of the core gene are the interactions among genes in the neighborhood of the core gene. Catenin (cadherin-associated protein), β1, 88 kDa (CTNNB1), phospholipase C, γ2 (phosphatidylinositol-specific) (PLCG2) and SRC were the 3 main central genes by degree, while integrin, β8 (ITGB8), PLCB1 and CNTNAP2 were the 3 main genes with the highest frequency of 4.

Discussion

Following the discovery of molecular target drugs, considerable developments have been achieved in treating malignant tumor. However, platinum based chemotherapy remains the main approach in several types of cancer. Resistance acquisition to cisplatin is one of the main problems of the treatment of all tumor types. In recent years, numerous studies have focused on cisplatin resistance and several mechanisms

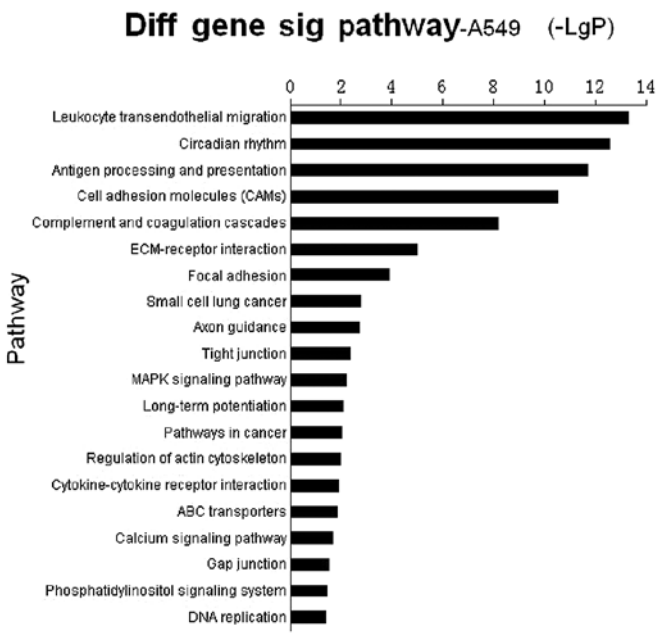

Figure 2. Histogram of signal pathways that were significantly different in A549/CDDP and A549. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

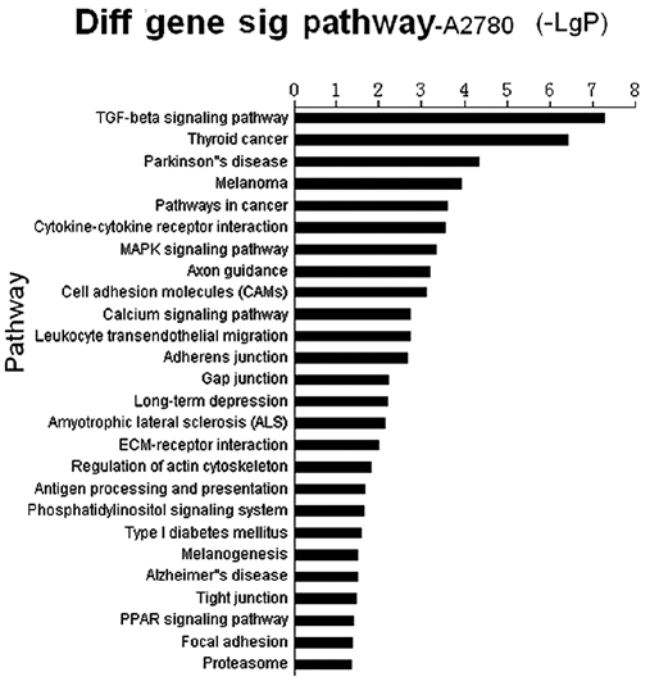

Figure 4. Histogram of signal pathways that were significantly different in A2780/CDDP and A2780. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

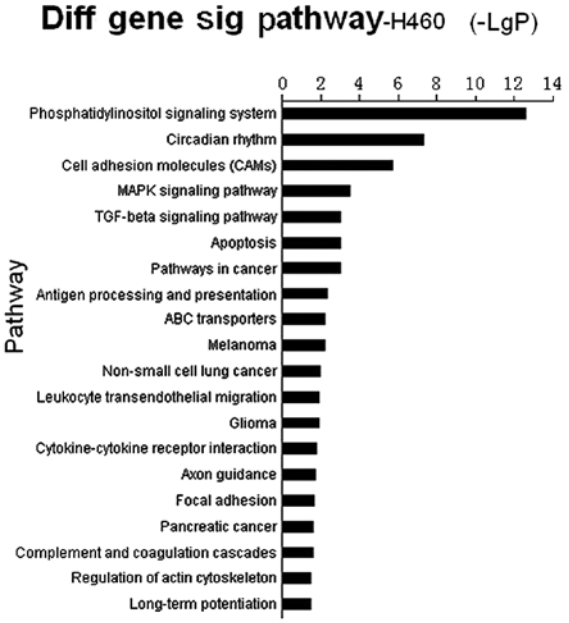

Figure 3. Histogram of signal pathways that were significantly different in H460/CDDP and H460. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

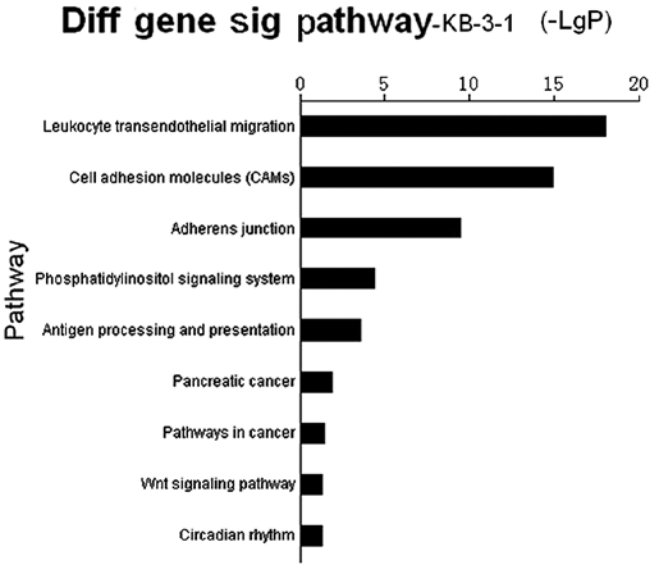

Figure 5. Histogram of signal pathways that were significantly different in KB-3-1/CDDP and KB-3-1. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

have been proposed. Stewart reviewed the mechanisms of resistance to cisplatin in 2007 by summarizing the 'classical' resistance mechanisms such as drug efflux and DNA repair, and presented some possible genes such as COX-2, epidermal growth factor (EGF) family that may relate to cisplatin resistance (12). Galluzzi *et al* (13) reviewed the molecular mechanisms of cisplatin resistance again. They classified the mechanisms into 4 alterations and proposed some genes that had been used in clinical chemotherapy prediction. In addition, although several other mechanisms such as microRNA and methylation on cisplatin resistance have previously been identified (14-17), their functions in mRNAs remain to be elucidated. Protein is the direct function target of cell behavior, and mRNA is a direct participant in coding protein. Although the roles of several genes in cisplatin resistance have been reported, there is still a lack of information regarding the general molecular mechanisms in different cancer cells. In this study, a gene expression signature for a subset of cancer

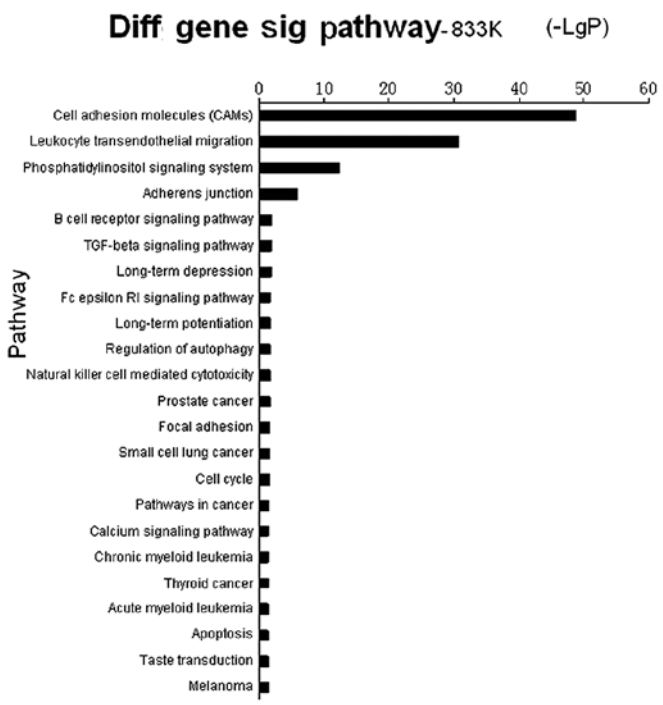

Figure 6. Histogram of signal pathways that were significantly different in 833K/CDDP and 833K. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

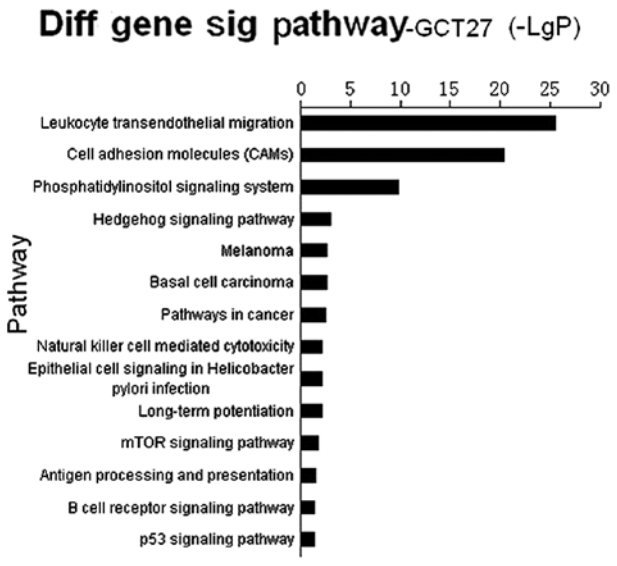

Figure 7. Histogram of signal pathways that were significantly different in GCT27/CDDP and GCT27. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

cell lines with resistance was established. The 7 cancer cell lines, which were from 4 types of malignant cancer including lung, ovary, testicular and oral cancer, were analyzed jointly. Thereby, our approach emphasizes the involvement of selected genes in general mechanisms of cisplatin resistance acquisition and avoids processes due to individual characteristics of a particular cell line. It is important to note that this is one of few studies on drug resistance that include more than 1 resistant cell line (18).

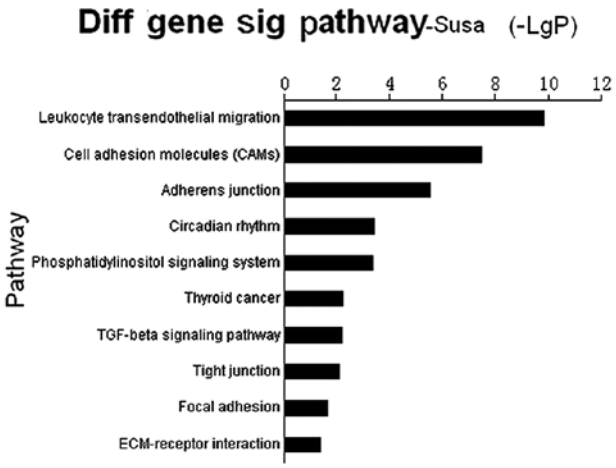

Figure 8. Histogram of signal pathways that were significantly different in Susa/CDDP and Susa. x-axis, negative logarithm of the P-value (-LgP); y-axis, the name of the pathway. The larger the -LgP, the smaller the P-value.

The present study followed up on microarray-based 7 pairs of cancer cell lines containing 4 types of cancer including NSCLC, ovarian, oral squamous and testicular cancer. All 7 pairs had a wild-type cell line and its cisplatin resistant variation cell line. To clarify molecular changes that may affect cisplatin resistance, we divided the cell lines into 2 groups to obtain cisplatin resistant-related differential expression genes. Expression of the upregulated gene number was in general slightly higher in the cisplatin-resistant group than in the wild-type group. Gene chips have become a useful tool for studying the development and progression of tumors owing to its high throughput, but it remains difficult to predict cancer cell drug resistance, mainly due to marked variation in the range of cisplatin resistance and the significant challenge in interpreting numerous complex data produced by the microarray (19) and determining the main genes responsible. The present study used the bioinformatics method to analyze functions and pathways of the differential expression genes, further clarifying their biological significance, and finally defining the key genes that affected the cisplatin resistance of 4 types of cancer.

For the microarray analyses, 7 cancer cell groups were analyzed on different types of gene chips and their intersection revealed no differentially expressed genes. We used another strategy to investigate their generality by studying their differential gene function at first and then by analysis of their mechanism based on function joint. We first obtained the differential gene function cluster by performing the GO-analysis and pathway analysis which were widely used in high throughput gene data analysis and then we merged these functional analysis results. Since the items in the GO database were numerous and repeated, we set the loose criteria as repetition more than 3 appearances in the 7 lists of GO analysis results. As we integrally considered the factors such as differential expressed genes including both up- and downregulated genes and their fold-changes when performing pathway analysis, we set the strict criteria as repetition frequency to more than half of the 7 lists.

In the latest reviews on molecular mechanisms of cisplatin resistance, the mechanisms were classified into 4 alterations

Table III.

GO term	GO ID	GO category	Regulated	No. of overlap
Biological process	GO:0043067	Regulation of programmed cell death	Down	4
	GO:0010941	Regulation of cell death	Down	4
	GO:0001568	Blood vessel development	Up	4
	GO:0009968	Negative regulation of signal transduction	Up	4
	GO:0022610	Biological adhesion	Up	4
	GO:0007155	Cell adhesion	Up	4
	GO:0001501	Skeletal system development	Up	4
	GO:0007267	Cell-cell signaling	Up	4
	GO:0043062	Extracellular structure organization	Up	4
	GO:0030182	Neuron differentiation	Down	3
	GO:0042127	Regulation of cell proliferation	Down	3
	GO:0042981	Regulation of apoptosis	Down	3
	GO:0051318	G1 phase	Down	3
	GO:0000904	Cell morphogenesis involved in differentiation	Down	3
	GO:0010627	Regulation of protein kinase cascade	Down	3
	GO:0006928	Cell motion	Down	3
	GO:0009611	Response to wounding	Up	3
	GO:0007389	Pattern specification process	Up	3
	GO:0001944	Vasculature development	Up	3
	GO:0010648	Negative regulation of cell communication	Up	3
	GO:0019226	Transmission of nerve impulse	Up	3
	GO:0043009	Chordate embryonic development	Up	3
	GO:0048598	Embryonic morphogenesis	Up	3
	GO:0048514	Blood vessel morphogenesis	Up	3
	GO:0009792	Embryonic development ending in birth or egg hatching	Up	3
	GO:0007507	Heart development	Up	3
	GO:0051056	Regulation of small GTPase mediated signal transduction	Up	3
	GO:0046578	Regulation of Ras protein signal transduction	Up	3
	GO:0043067	Regulation of programmed cell death	Up	3
	GO:0008219	Cell death	Up	3
	GO:0030199	Collagen fibril organization	Up	3
	GO:0010941	Regulation of cell death	Up	3
	GO:0016265	Death	Up	3
	GO:0042981	Regulation of apoptosis	Up	3
	GO:0030182	Neuron differentiation	Up	3
	GO:0055114	Oxidation reduction	Up	3
	GO:0009991	Response to extracellular stimulus	Up	3
	GO:0031667	Response to nutrient levels	Up	3
	GO:0043068	Positive regulation of programmed cell death	Up	3
	GO:0010942	Positive regulation of cell death	Up	3
	GO:0043065	Positive regulation of apoptosis	Up	3
Cellular component	GO:0044421	Extracellular region part	Up	5
	GO:0044459	Plasma membrane part	Up	5
	GO:0005626	Insoluble fraction	Up	5
	GO:0005624	Membrane fraction	Up	5
	GO:0000267	Cell fraction	Up	5
	GO:0042995	Cell projection	Up	4
	GO:0045202	Synapse	Up	4
	GO:0005911	Cell-cell junction	Up	4
	GO:0019898	Extrinsic to membrane	Up	4
	GO:0005886	Plasma membrane	Up	3
	GO:0030054	Cell junction	Up	3
	GO:0031012	Extracellular matrix	Up	3
	GO:0031226	Intrinsic to plasma membrane	Up	3

Table III. Continued.

GO term	GO ID	GO category	Regulated	No. of overlap
	GO:0005887	Integral to plasma membrane	Up	3
	GO:0005578	Proteinaceous extracellular matrix	Up	3
	GO:0005794	Golgi apparatus	Up	3
	GO:0005615	Extracellular space	Up	3
	GO:0005856	Cytoskeleton	Up	3
	GO:0044456	Synapse part	Up	3
	GO:0009986	Cell surface	Up	3
	GO:0016324	Apical plasma membrane	Up	3
	GO:0005887	Integral to plasma membrane	Down	3
	GO:0000267	Cell fraction	Down	3
	GO:0031981	Nuclear lumen	Down	3
	GO:0043233	Organelle lumen	Down	3
	GO:0044459	Plasma membrane part	Down	3
	GO:0030424	Axon	Down	3
	GO:0031226	Intrinsic to plasma membrane	Down	3
	GO:0031974	Membrane-enclosed lumen	Down	3
	GO:0070013	Intracellular organelle lumen	Down	3
	GO:0043232	Intracellular non-membrane-bounded organelle	Down	3
	GO:0043228	Non-membrane-bounded organelle	Down	3
	GO:0009986	Cell surface	Down	3
	GO:0044432	Endoplasmic reticulum part	Down	3
Molecular function	GO:0008092	Cytoskeletal protein binding	Up	4
	GO:0003779	Actin binding	Up	4
	GO:0030247	Polysaccharide binding	Up	4
	GO:0001871	Pattern binding	Up	4
	GO:0042802	Identical protein binding	Down	3
	GO:0016564	Transcription repressor activity	Down	3
	GO:0046983	Protein dimerization activity	Up	3
	GO:0042802	Identical protein binding	Up	3
	GO:0008289	Lipid binding	Up	3
	GO:0042803	Protein homodimerization activity	Up	3

GO, Gene Ontology.

Table IV.

KEGG term	KEGG pathway	No. of overlap
04670	Leukocyte transendothelial migration	7
04070	Phosphatidylinositol signaling system	7
04514	Cell adhesion molecules (CAMs)	7
05200	Pathways in cancer	6
04612	Antigen processing and presentation	5
04510	Focal adhesion	5
04720	Long-term potentiation	4
04710	Circadian rhythm	4
04350	TGF-β signaling pathway	4
05218	Melanoma	4
04520	Adherens junction	4

TGF-β, transforming growth factor-β.

including the binding of cisplatin to DNA, direct relation to DNA-cisplatin adducts, the lethal signaling pathway and molecular circuitries that do not present obvious links with cisplatin-elicited signals (13). The GO is widely recognized as the leading tool for the organization and functional annotation of molecular aspect (20). GO analysis was used to interpret each GO of differential expressed gene and analyzed it statistically. By using the criteria of $P<0.05$, significant GOs and genes involved in them were obtained. GO terms regarding programmed cell death in the biological process section plays the most important role in cisplatin resistance, this is easily understood as several genes have been reported to affect cisplatin resistance by participating in lethal signaling pathways elicited by cisplatin-mediated DNA damage (21-23). Cell adhesion is another group of items that markedly differently expressed in the merged lists. Dexamethasone has been reported to enhance cell resistance to chemotherapy by increasing adhesion to extracellular matrix in human ovarian cancer cells (24). In the cellular component part, membrane-related items such as

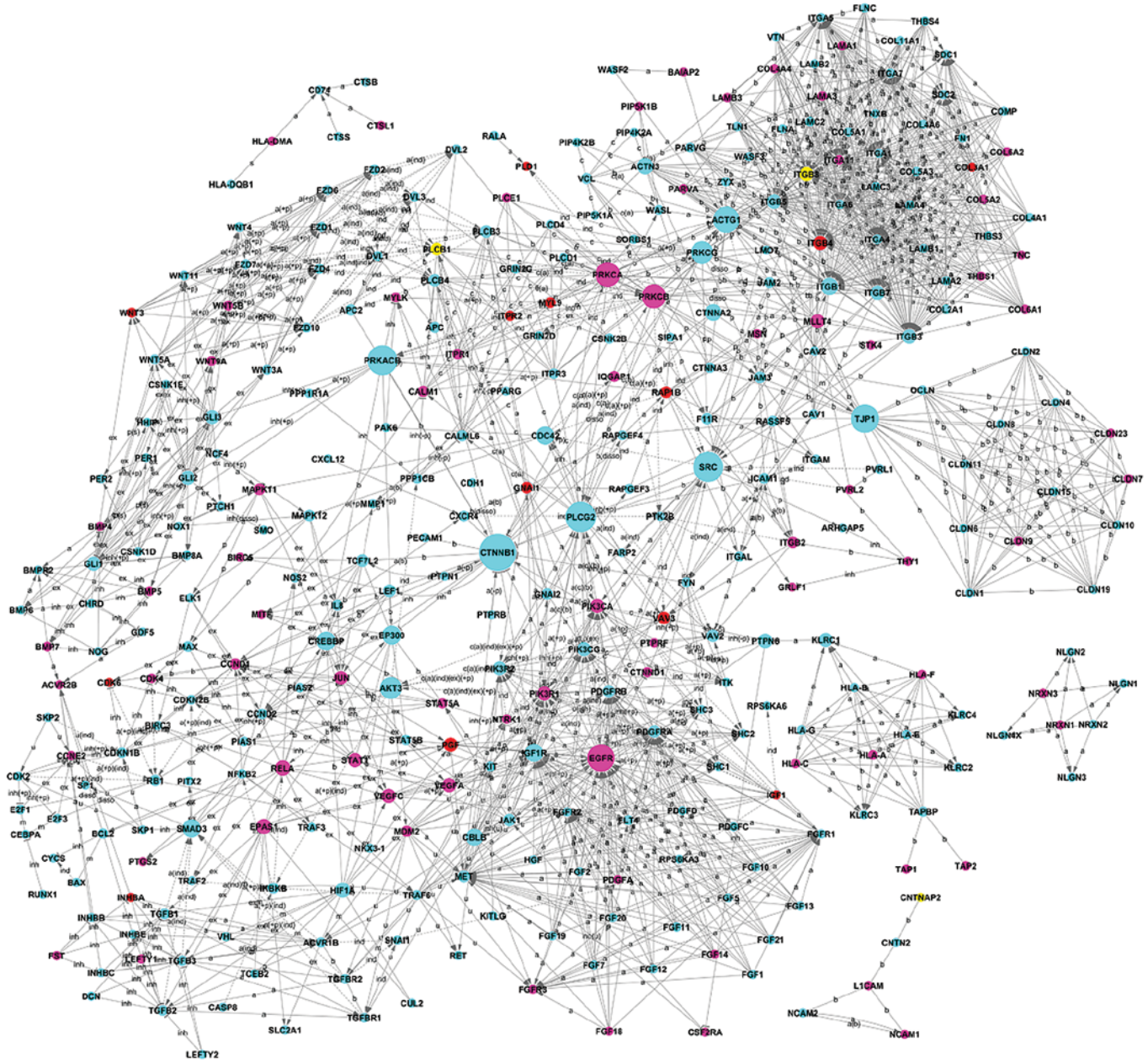

Figure 9. Signal transduction networks of cisplatin-resistant-related genes. Circles represent genes. Repetition frequencies of genes that appear in 7 groups are represented by different colors. Green, pink, red and yellow indicate the repetition frequency of genes as 1, 2, 3 and 4, respectively. Arrow represents the activation of (a); straight line represents combination; dotted line represents indirect effects; annotation of relation attributes are listed in Table V in detail.

membrane fraction and cell-cell junction altered significantly indicates molecules affect cisplatin resistance through cell membrane to a large extent. RhoA, ion pumps and membrane lipid have been reported to impact the cell properties of drug resistance (25-27). Several protein binding character changes in the molecular function portion also reveal it may be a result of alteration of binding cisplatin to DNA. Based on this, we hypothesized that the other items listed also have a function, which remains unclear, on cisplatin resistance.

GO analysis is a classical method to annotate gene function but remains inexact in some fields. Pathway analysis can show the distinct biological process and can find significant pathways that differential expression genes participate in, based on which we can have a comprehensive understanding of the interactions of genes, functions that they participate in and relations between upstream and downstream, and obtain genes involved in these significant pathways. Appearance of pathways on phosphatidylinositol signaling system, cell adhesion molecules and pathways in cancer confirm their concordance with GO terms and their critical role in cisplatin resistance. Numerous studies had proved that the PI3K/Akt signaling pathway, which belongs to the phosphatidylinositol signaling pathway, is involved in different cancer cell cisplatin resistance (28-30). The role of cell adhesion molecules has been discussed in the GO analysis part. Adherens junction protein γ-catenin was found downregulated and altered localization in cisplatin-resistant adenocarcinoma cells (31). Numerous signaling pathways such as MAPK, vascular endothelial growth factor (VEGF) and p53 involved in pathways in cancer have been reported in cisplatin resistance (32-34).

Table V.

A, Gene features

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
CTNNB1	Catenin (cadherin-associated protein), β1, 88 kDa	0.999996011	27	21	14	1
PLCG2	Phospholipase C, γ2 (phosphatidylinositol-specific)	0.753865101	24	16	11	1
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	0.742746802	23	13	10	1
PRKACB	Protein kinase, cAMP-dependent, catalytic, β	0.735692693	15	3	12	1
TJP1	Tight junction protein 1 (zona occludens 1)	0.67725436	24	24	21	1
EGFR	Epidermal growth factor receptor	0.642287321	41	26	15	2
ACTG1	Actin, γ1	0.6062699	32	32	21	1
PRKCA	Protein kinase C, α	0.548143502	28	16	27	2
PRKCB	Protein kinase C, β	0.529237948	28	16	24	2
PRKCG	Protein kinase C, γ	0.466543012	25	16	24	1
AKT3	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, γ)	0.445875453	10	5	6	1
CREBBP	CREB binding protein	0.346819878	12	10	9	1
EP300	E1A binding protein p300	0.346819878	12	10	9	1
CDC42	Cell division cycle 42 (GTP binding protein, 25 kDa)	0.322755868	11	6	5	1
SMAD3	SMAD family member 3	0.322045544	12	7	5	1
IGF1R	Insulin-like growth factor 1 receptor	0.313437547	35	24	11	1
CBLB	Cbl proto-oncogene, E3 ubiquitin protein ligase B	0.285851733	20	1	19	1
ITGB1	Integrin, β1 (fibronectin receptor, β polypeptide, antigen CD29 includes MDF2, MSK12)	0.284749522	44	43	14	1
CTNNA2	Catenin (cadherin-associated protein), α2	0.263939296	7	7	7	1
ACTN3	Actinin, α3	0.251235683	22	22	22	1
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	0.226235037	14	2	12	2
HIF1A	Hypoxia inducible factor 1, α subunit (basic helix-loop-helix transcription factor)	0.212984985	12	5	12	1
EPAS1	Endothelial PAS domain protein 1	0.212984985	12	5	12	2
PTPN6	Protein tyrosine phosphatase, non-receptor type 6	0.191058135	6	1	5	1
ITGB4	Integrin, β4	0.181942849	41	40	13	3
ITGB5	Integrin, β5	0.181942849	41	40	13	1
ITGB3	Integrin, β3 (platelet glycoprotein IIIa, antigen CD61)	0.181942849	41	40	13	1
ITGB8	Integrin, β8	0.181942849	41	40	13	4
ITGB7	Integrin, β7	0.181942849	41	40	13	1
GLI2	GLI family zinc finger 2	0.178435676	21	5	16	1
GLI3	GLI family zinc finger 3	0.178435676	21	5	16	1
GLI1	GLI family zinc finger 1	0.178435676	21	5	16	1
PGF	Placental growth factor	0.177790774	11	5	6	3
VEGFC	Vascular endothelial growth factor C	0.177790774	11	5	6	2
VEGFA	Vascular endothelial growth factor A	0.177790774	11	5	6	2
MLLT4	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 4	0.176392878	12	11	12	2

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
STAT1	Signal transducer and activator of transcription 1, 91 kDa	0.175849881	10	5	7	2
PIK3CA	Phosphoinositide-3-kinase, catalytic, α polypeptide	0.174636842	21	18	5	2
PIK3CG	Phosphoinositide-3-kinase, catalytic, γ polypeptide	0.174636842	21	18	5	1
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (α)	0.174636842	21	18	5	2
PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2 (β)	0.174636842	21	18	5	1
KLRC1	Killer cell lectin-like receptor 1 subfamily C, member	0.172726882	7	6	1	1
JUN	Jun proto-oncogene	0.156150273	12	4	8	2
LEF1	Lymphoid enhancer-binding factor 1	0.126218368	9	4	8	1
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	0.126218368	9	4	8	1
PLCB3	Phospholipase C, β 3 (phosphatidylinositol-specific)	0.125121422	16	15	8	1
PLCB4	Phospholipase C, β 4	0.125121422	16	15	8	1
PLCB1	Phospholipase C, β 1 (phosphoinositide-specific)	0.125121422	16	15	8	4
PDGFRA	Platelet-derived growth factor receptor, α polypeptide	0.120935017	33	24	9	1
VAV3	Vav 3 guanine nucleotide exchange factor	0.114910435	11	10	5	3
VAV2	Vav 2 guanine nucleotide exchange factor	0.114910435	11	10	5	1
JAK1	Janus kinase 1	0.114890597	11	4	7	1
PDGFRB	Platelet-derived growth factor receptor, β polypeptide	0.111719369	30	21	9	1
MET	Met proto-oncogene (hepatocyte growth factor receptor)	0.102073555	33	24	9	1
RAP1B	RAP1B, member of RAS oncogene family	0.095616574	11	4	8	3
GNAI2	Guanine nucleotide binding protein (G protein), α inhibiting activity polypeptide 2	0.093690523	11	2	10	1
MAPK11	Mitogen-activated protein kinase 11	0.093494671	7	3	4	2
MAPK12	Mitogen-activated protein kinase 12	0.093494671	7	3	4	1
GNAI1	Guanine nucleotide binding protein (G protein), α inhibiting activity polypeptide 1	0.090079268	11	2	9	3
JAM3	Junctional adhesion molecule 3	0.089930583	9	9	6	1
F11R	F11 receptor	0.086027526	9	9	6	1
RB1	Retinoblastoma 1	0.082364122	9	9	2	1
ITGB2	Integrin, β2 (complement component 3 receptor 3 and 4 subunit)	0.080583097	10	6	7	2
CCND1	Cyclin D1	0.078908556	16	15	3	2
MDM2	Mdm2, p53 E3 ubiquitin protein ligase homolog (mouse)	0.075153331	14	1	13	2
CALM1	Calmodulin 1 (phosphorylase kinase, δ)	0.074699383	12	11	10	2
CALML6	Calmodulin-like 6	0.074699383	12	11	10	1
APC2	Adenomatous polyposis coli 2	0.072497133	4	4	4	1
APC	Adenomatous polyposis coli	0.072497133	4	4	4	1
VCL	Vinculin	0.070911065	10	10	10	1
CXCR4	Chemokine (C-X-C motif) receptor 4	0.065869722	5	3	3	1
IKBKB	Inhibitor of κ light polypeptide gene enhancer in B-cells, kinase β	0.06194965	7	5	2	1
RASSF5	Ras association (RalGDS/AF-6) domain family member 5	0.060022707	8	3	6	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
JAM2	Junctional adhesion molecule 2	0.059645234	9	9	6	1
MYL9	Myosin, light chain 9, regulatory	0.059581214	4	3	2	3
IQGAP1	IQ motif containing GTPase activating protein 1	0.058424766	3	1	2	2
ICAM1	Intercellular adhesion molecule 1	0.057953639	7	4	4	1
TGFB3	Transforming growth factor, β3	0.056454983	13	9	4	1
TGFB1	Transforming growth factor, β1	0.056454983	13	9	4	1
TGFB2	Transforming growth factor, β2	0.056454983	13	9	4	1
MAX	MYC associated factor X	0.054542012	7	2	5	1
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.049795585	11	6	6	1
ITPR3	Inositol 1,4,5-trisphosphate receptor, type 3	0.047992954	13	13	12	1
ITPR1	Inositol 1,4,5-trisphosphate receptor, type 1	0.047992954	13	13	12	2
ITPR2	Inositol 1,4,5-trisphosphate receptor, type 2	0.047992954	13	13	12	3
IL8	Interleukin 8	0.046078769	5	4	1	1
Bcl-2	B-cell CLL/lymphoma 2	0.044580232	4	3	2	1
CSNK2B	Casein kinase 2, β polypeptide	0.044523355	5	3	5	1
ACVR1B	Activin A receptor, type IB	0.043802525	13	11	3	1
CCND2	Cyclin D2	0.04296769	11	10	1	1
CTNNA3	Catenin (cadherin-associated protein), α3	0.042051264	6	5	6	1
TAPBP	TAP binding protein (tapasin)	0.041309867	8	8	8	1
CTNND1	Catenin (cadherin-associated protein), δ1	0.040164917	5	4	1	2
PPP1CB	Protein phosphatase 1, catalytic subunit, β isozyme	0.036670307	5	2	3	1
FZD1	Frizzled family receptor 1	0.035644104	13	7	6	1
FZD2	Frizzled family receptor 2	0.035644104	13	7	6	1
FZD4	Frizzled family receptor 4	0.035644104	13	7	6	1
FZD7	Frizzled family receptor 7	0.035644104	13	7	6	1
FZD6	Frizzled family receptor 6	0.035644104	13	7	6	1
FZD10	Frizzled family receptor 10	0.035644104	13	7	6	1
FYN	FYN oncogene related to SRC, FGR, YES	0.0342385	9	3	6	1
TRAF6	TNF receptor-associated factor 6, E3 ubiquitin protein ligase	0.034231141	16	2	14	1
RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	0.034039755	4	2	2	1
RAPGEF3	Rap guanine nucleotide exchange factor (GEF) 3	0.034039755	4	2	2	1
STAT5A	Signal transducer and activator of transcription 5A	0.033220221	9	7	4	2
STAT5B	Signal transducer and activator of transcription 5B	0.033220221	9	7	4	1
WNT9A	Wingless-type MMTV integration site family, member 9A	0.030564384	9	3	6	2
WNT3A	Wingless-type MMTV integration site family, member 3A	0.030564384	9	3	6	1
WNT4	Wingless-type MMTV integration site family, member 4	0.030564384	9	3	6	1
WNT3	Wingless-type MMTV integration site family, member 3	0.030564384	9	3	6	3
WNT5A	Wingless-type MMTV integration site family, member 5A	0.030564384	9	3	6	1
WNT5B	Wingless-type MMTV integration site family, member 5B	0.030564384	9	3	6	2
WNT11	Wingless-type MMTV integration site family, member 11	0.030564384	9	3	6	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
WASL	Wiskott-Aldrich syndrome-like	0.029983832	5	4	1	1
ITGAL	Integrin, α L (antigen CD11A (p180), lymphocyte function-associated antigen 1; α polypeptide)	0.02927462	8	4	5	1
CAV1	Caveolin 1, caveolae protein, 22 kDa	0.02708201	8	7	7	1
BMP7	Bone morphogenetic protein 7	0.026432577	7	5	2	2
ITGAM	Integrin, α M (complement component 3 receptor 3 subunit)	0.026106042	5	4	3	1
DVL2	Dishevelled, dsh homolog 2 (*Drosophila*)	0.02587891	9	9	3	1
DVL3	Dishevelled, dsh homolog 3 (*Drosophila*)	0.02587891	9	9	3	1
DVL1	Dishevelled, dsh homolog 1 (*Drosophila*)	0.02587891	9	9	3	1
ITGA4	Integrin, α4 (antigen CD49D, α4 subunit of VLA-4 receptor)	0.024940917	38	38	8	1
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	0.022679014	10	4	6	1
MYLK	Myosin light chain kinase	0.022365192	5	4	1	2
PTCH1	Patched 1	0.022290116	4	3	1	1
E2F1	E2F transcription factor 1	0.021004381	4	3	3	1
E2F3	E2F transcription factor 3	0.021004381	4	3	3	1
TGFBR2	Transforming growth factor, β receptor II (70/80 kDa)	0.020415116	10	8	4	1
FLT4	fms-related tyrosine kinase 4	0.020317237	15	8	7	1
NOS2	Nitric oxide synthase 2, inducible	0.020297548	4	4	2	1
TGFBR1	Transforming growth factor, β receptor 1	0.020242992	9	7	3	1
SORBS1	Sorbin and SH3 domain containing 1	0.020153611	3	3	2	1
TLN1	Talin 1	0.019788076	13	13	13	1
CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	0.019595116	10	6	4	1
NFKB2	Nuclear factor of κ light polypeptide gene enhancer in B-cells 2 (p49/p100)	0.017309379	9	1	8	1
CAV2	Caveolin 2	0.016399739	7	6	7	1
OCLN	Occludin	0.013818722	14	14	13	1
PIAS2	Protein inhibitor of activated STAT, 2	0.012519431	5	1	4	1
PIAS1	Protein inhibitor of activated STAT, 1	0.012519431	5	1	4	1
PVRL1	Poliovirus receptor-related 1 (herpesvirus entry mediator C)	0.012012429	3	2	3	1
PVRL2	Poliovirus receptor-related 2 (herpesvirus entry mediator B)	0.012012429	3	2	3	2
PARVG	Parvin, γ	0.011249328	13	13	13	1
FLNC	Filamin C, γ	0.011249328	12	12	12	1
FLNA	Filamin A, α	0.011249328	12	12	12	1
PARVA	Parvin, α	0.011249328	13	13	13	2
CCNE2	Cyclin E2	0.010522022	7	5	2	2
HLA-A	Major histocompatibility complex, class I, A	0.009768062	10	6	10	2
HLA-C	Major histocompatibility complex, class I, C	0.009768062	10	6	10	2
HLA-B	Major histocompatibility complex, class I, B	0.009768062	10	6	10	1
HLA-E	Major histocompatibility complex, class I, E	0.009768062	10	6	10	1
HLA-G	Major histocompatibility complex, class I, G	0.009768062	10	6	10	1
HLA-F	Major histocompatibility complex, class I, F	0.009768062	10	6	10	2
ELK1	ELK1, member of ETS oncogene family	0.009089382	5	2	3	1
PTK2B	PTK2B protein tyrosine kinase 2β	0.008746784	7	5	2	1
ITGA11	Integrin, α11	0.006879865	35	35	7	2
ITGA1	Integrin, α1	0.006879865	35	35	7	1
ITGA6	Integrin, α6	0.006879865	35	35	7	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
ITGA5	Integrin, α5 (fibronectin receptor, α polypeptide)	0.006879865	35	35	7	1
ITGA7	Integrin, α7	0.006879865	35	35	7	1
FGFR1	Fibroblast growth factor receptor 1	0.006825859	23	18	5	1
NTRK1	Neurotrophic tyrosine kinase, receptor, type 1	0.00657735	11	3	8	2
PPP1R1A	Protein phosphatase 1, regulatory (inhibitor) subunit 1A	0.006156131	2	1	1	1
FGFR2	Fibroblast growth factor receptor 2	0.005994849	24	20	4	1
INHBB	Inhibin, βB	0.004776453	6	1	5	1
INHBA	Inhibin, βA	0.004776453	6	1	5	3
INHBE	Inhibin, βE	0.004776453	6	1	5	1
INHBC	Inhibin, βC	0.004776453	6	1	5	1
BMP4	Bone morphogenetic protein 4	0.004486711	6	5	1	2
BMP5	Bone morphogenetic protein 5	0.004486711	6	5	1	2
BMP8A	Bone morphogenetic protein 8a	0.004486711	6	5	1	1
BMP6	Bone morphogenetic protein 6	0.004486711	6	5	1	1
CDK4	Cyclin-dependent kinase 4	0.004121372	6	5	2	2
CDK2	Cyclin-dependent kinase 2	0.003937999	7	3	5	1
CDK6	Cyclin-dependent kinase 6	0.003521631	5	4	2	3
MSN	Moesin	0.003453132	2	1	2	2
GRIN2C	Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	0.003410414	5	5	2	1
GRIN2D	Glutamate receptor, ionotropic, N-methyl D-aspartate 2D	0.003410414	5	5	2	1
THY1	Thy-1 cell surface antigen	0.003181286	4	2	4	2
PAK6	p21 protein (Cdc42/Rac)-activated kinase 6	0.00297153	2	1	1	1
PIP4K2B	Phosphatidylinositol-5-phosphate 4-kinase, type II, β	0.002788191	3	2	3	1
PIP5K1B	Phosphatidylinositol-4-phosphate 5-kinase, type I, β	0.002788191	3	2	3	2
PIP5K1A	Phosphatidylinositol-4-phosphate 5-kinase, type I, α	0.002788191	3	2	3	1
LMO7	LIM domain 7	0.001459619	2	2	2	1
CEBPA	CCAAT/enhancer binding protein (C/EBP), α	0.000946684	3	3	2	1
L1CAM	L1 cell adhesion molecule	0.000688498	3	3	3	2
CNTN2	Contactin 2 (axonal)	0.000516373	2	2	2	1
TRAF2	TNF receptor-associated factor 2	0.000286874	3	2	1	1
GDF5	Growth differentiation factor 5	0.00011475	4	2	2	1
PLCE1	Phospholipase C, ε1	8.80837E-05	6	6	6	2
PLCD4	Phospholipase C, δ4	8.80837E-05	6	6	6	1
PLCD1	Phospholipase C, δ1	8.80837E-05	6	6	6	1
HLA-DMA	Major histocompatibility complex, class II, DM α	8.60622E-05	2	1	2	2
CUL2	Cullin 2	3.44249E-05	2	2	2	1
TCEB2	Transcription elongation factor B (SIII), polypeptide 2 (18 kDa, elongin B)	3.44249E-05	2	2	2	1
VHL	Von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	3.44249E-05	2	2	2	1
KLRC4	Killer cell lectin-like receptor subfamily C, member 4	0	6	6	0	1
KLRC2	Killer cell lectin-like receptor subfamily C, member 2	0	6	6	0	1
NOG	Noggin	0	6	0	6	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
KLRC3	Killer cell lectin-like receptor subfamily C, member 3	0	6	6	0	1
PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	0	2	2	0	2
FST	Follistatin	0	4	0	4	2
PITX2	Paired-like homeodomain 2	0	1	1	0	1
PLD1	Phospholipase D1, phosphatidylcholine-specific	0	3	3	0	3
PTPRF	Protein tyrosine phosphatase, receptor type, F	0	4	0	4	2
NCF4	Neutrophil cytosolic factor 4, 40 kDa	0	2	0	2	1
SKP2	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase	0	3	0	3	1
SKP1	S-phase kinase-associated protein 1	0	4	0	4	1
FARP2	FERM, RhoGEF and pleckstrin domain protein 2	0	2	1	1	1
LAMC3	Laminin, γ3	0	12	0	12	1
LAMC2	Laminin, γ2	0	12	0	12	1
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	0	4	4	0	1
CTSL1	Cathepsin L1	0	1	0	1	2
LAMB3	Laminin, β3	0	12	0	12	2
LAMB2	Laminin, β2 (laminin S)	0	12	0	12	1
LAMB1	Laminin, β1	0	12	0	12	1
HGF	Hepatocyte growth factor (hepapoietin A; scatter factor)	0	7	0	7	1
LAMA2	Laminin, α2	0	12	0	12	1
LAMA1	Laminin, α1	0	12	0	12	2
LAMA4	Laminin, α4	0	12	0	12	1
LAMA3	Laminin, α3	0	12	0	12	2
NLGN4X	Neuroligin 4, X-linked	0	3	3	0	1
FGF5	Fibroblast growth factor 5	0	8	0	8	1
FGF7	Fibroblast growth factor 7	0	8	0	8	1
WASF3	WAS protein family, member 3	0	1	0	1	1
PPARG	Peroxisome proliferator-activated receptor γ	0	2	0	2	1
MITF	Microphthalmia-associated transcription factor	0	4	4	0	2
WASF2	WAS protein family, member 2	0	2	1	1	1
HHIP	Hedgehog interacting protein	0	3	3	0	1
FGF1	Fibroblast growth factor 1 (acidic)	0	8	0	8	1
COL11A1	Collagen, type XI, α1	0	14	0	14	1
FGF2	Fibroblast growth factor 2 (basic)	0	8	0	8	1
HLA-DQB1	Major histocompatibility complex, class II, DQ β1	0	1	1	1	1
CLDN19	Claudin 19	0	13	13	13	1
COL3A1	Collagen, type III, α1	0	14	0	14	3
COL2A1	Collagen, type II, α1	0	14	0	14	1
CLDN10	Claudin 10	0	13	13	13	1
CLDN11	Claudin 11	0	13	13	13	1
CLDN15	Claudin 15	0	13	13	13	1
PER2	Period homolog 2 (*Drosophila*)	0	3	3	1	1
COL6A2	Collagen, type VI, α2	0	14	0	14	2
PER1	Period homolog 1 (*Drosophila*)	0	3	3	1	1
COL6A1	Collagen, type VI, α1	0	14	0	14	2
THBS1	Thrombospondin 1	0	14	0	14	2
THBS3	Thrombospondin 3	0	14	0	14	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
THBS4	Thrombospondin 4	0	14	0	14	1
NLGN1	Neuroligin 1	0	3	3	0	1
NLGN2	Neuroligin 2	0	3	3	0	1
IGF1	Insulin-like growth factor 1 (somatomedin C)	0	14	0	14	3
NLGN3	Neuroligin 3	0	3	3	0	1
BIRC5	Baculoviral IAP repeat containing 5	0	2	2	0	2
BIRC3	Baculoviral IAP repeat containing 3	0	3	3	0	1
SNAI1	Snail homolog 1 (*Drosophila*)	0	4	4	0	1
CLDN23	Claudin 23	0	13	13	13	2
RPS6KA6	Ribosomal protein S6 kinase, 90 kDa, polypeptide 6	0	1	1	0	1
RPS6KA3	Ribosomal protein S6 kinase, 90 kDa, polypeptide 3	0	1	1	0	1
CSNK1D	Casein kinase 1, δ	0	6	1	6	1
CSNK1E	Casein kinase 1, ε	0	6	1	6	1
FGF19	Fibroblast growth factor 19	0	8	0	8	1
FGF18	Fibroblast growth factor 18	0	8	0	8	2
PDGFA	Platelet-derived growth factor α polypeptide	0	8	0	8	2
FGF14	Fibroblast growth factor 14	0	8	0	8	2
FGF11	Fibroblast growth factor 11	0	8	0	8	1
FGF10	Fibroblast growth factor 10	0	8	0	8	1
VTN	Vitronectin	0	12	0	12	1
FGF13	Fibroblast growth factor 13	0	8	0	8	1
FGF12	Fibroblast growth factor 12	0	8	0	8	1
CXCL12	Chemokine (C-X-C motif) ligand 12	0	1	0	1	1
MMP1	Matrix metallopeptidase 1 (interstitial collagenase)	0	2	2	0	1
SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	0	2	2	0	1
RALA	v-ral simian leukemia viral oncogene homolog A (ras related)	0	1	0	1	1
PDGFC	Platelet derived growth factor C	0	8	0	8	1
PDGFD	Platelet derived growth factor D	0	8	0	8	1
RET	Ret proto-oncogene	0	3	3	0	1
BAIAP2	BAI1-associated protein 2	0	2	0	2	2
FGF21	Fibroblast growth factor 21	0	8	0	8	1
FGF20	Fibroblast growth factor 20	0	8	0	8	1
STK4	Serine/threonine kinase 4	0	1	1	1	2
NCAM1	Neural cell adhesion molecule 1	0	3	2	2	2
NCAM2	Neural cell adhesion molecule 2	0	3	2	2	1
FGFR3	Fibroblast growth factor receptor 3	0	16	16	0	2
SIPA1	Signal-induced proliferation-associated 1	0	1	1	0	1
BMPR2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	0	6	6	0	1
NKX3-1	NK3 homeobox 1	0	1	1	1	1
RUNX1	Runt-related transcription factor 1	0	1	0	1	1
TRAF3	TNF receptor-associated factor 3	0	2	2	0	1
FN1	Fibronectin 1	0	14	0	14	1
ITK	IL2-inducible T-cell kinase	0	6	4	2	1
PTPN1	Protein tyrosine phosphatase, non-receptor type 1	0	1	0	1	1
PIP4K2A	Phosphatidylinositol-5-phosphate 4-kinase, type II, α	0	2	2	2	1
CHRD	Chordin	0	6	0	6	1

Table V-A. Continued.

Gene symbol	Description	Betweenness centrality	Degree	Indegree	Outdegree	Frequency
CLDN8	Claudin 8	0	13	13	13	1
CLDN7	Claudin 7	0	13	13	13	2
CLDN9	Claudin 9	0	13	13	13	2
CLDN4	Claudin 4	0	13	13	13	1
CLDN6	Claudin 6	0	13	13	13	1
SDC2	Syndecan 2	0	18	18	0	1
ARHGAP5	Rho GTPase activating protein 5	0	2	2	0	1
CASP8	Caspase 8, apoptosis-related cysteine peptidase	0	1	0	1	1
CNTNAP2	Contactin associated protein-like 2	0	1	1	1	4
SHC1	SHC (Src homology 2 domain containing) transforming protein 1	0	9	9	0	1
ZYX	Zyxin	0	2	2	2	1
SHC3	SHC (Src homology 2 domain containing) transforming protein 3	0	9	9	0	1
SHC2	SHC (Src homology 2 domain containing) transforming protein 2	0	9	9	0	1
CSF2RA	Colony stimulating factor 2 receptor, α, low-affinity (granulocyte-macrophage)	0	3	2	1	2
NRXN2	Neurexin 2	0	4	0	4	1
NRXN3	Neurexin 3	0	4	0	4	2
CYCS	Cytochrome *c*, somatic	0	2	2	0	1
NRXN1	Neurexin 1	0	4	0	4	2
CTSS	Cathepsin S	0	1	0	1	1
LEFTY1	Left-right determination factor 1	0	3	0	3	2
SMO	Smoothened, frizzled family receptor	0	1	1	0	1
SDC1	Syndecan 1	0	18	18	0	1
ACVR2B	Activin A receptor, type IIB	0	6	6	0	2
CLDN1	Claudin 1	0	13	13	13	1
CLDN2	Claudin 2	0	13	13	13	1
CTSB	Cathepsin B	0	1	0	1	1
TNC	Tenascin C	0	14	0	14	2
KITLG	KIT ligand	0	1	0	1	1
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	0	1	0	1	1
DCN	Decorin	0	3	0	3	1
TAP2	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	0	1	1	1	2
COMP	Cartilage oligomeric matrix protein	0	14	0	14	1
LEFTY2	Left-right determination factor 2	0	3	0	3	1
TAP1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0	1	1	1	2
COL4A4	Collagen, type IV, α 4	0	14	0	14	2
PTPRB	Protein tyrosine phosphatase, receptor type, B	0	1	0	1	1
TNXB	Tenascin XB	0	14	0	14	1
COL4A1	Collagen, type IV, α1	0	14	0	14	1
NOX1	NADPH oxidase 1	0	2	0	2	1
COL5A3	Collagen, type V, α3	0	14	0	14	1
COL5A2	Collagen, type V, α2	0	14	0	14	2
COL5A1	Collagen, type V, α1	0	14	0	14	1
COL4A6	Collagen, type IV, α6	0	14	0	14	1
SP1	Sp1 transcription factor	0	2	1	1	1
BAX	Bcl-2-associated X protein	0	2	1	2	1
PECAM1	Platelet/endothelial cell adhesion molecule 1	0	1	1	1	1
GRLF1	Glucocorticoid receptor DNA binding factor 1	0	2	2	0	2

Table V. Continued.

B, Annotation	
Subtype_name	jx
Activation (binding/association)	a
Inhibition (dissociation)	inh(disso)
Activation	a
Dissociation	disso
Binding/association	b
Indirect effect	ind
Activation (phosphorylation)	a(+p)
Compound	c
Ubiquitination	u
Missing interaction	m
Compound (activation) (phosphorylation) (indirect effect) (expression)	c(a)(ind)(ex)(+p)
Phosphorylation	p
Inhibition	inh
Expression	ex
Compound (activation)	c(a)
Inhibition (dephosphorylation)	inh(-p)
Activation (indirect effect)	a(ind)
Inhibition (phosphorylation)	inh(+p)
Ubiquitination (inhibition)	u(ind)
Inhibition (ubiquitination)	inh(u)
State change	s
Binding/association (dissociation)	b(disso)
Activation (dephosphorylation)	a(-p)
Activation (binding/association)	a(b)
Compound (expression)	c(ex)
Ubiquitination (inhibition)	u(inh)
Activation (phosphorylation) (indirect effect)	a(+p)(ind)
Activation (ubiquitination)	a(u)
Phosphorylation (state change)	p(s)
Activation (binding/association) (compound)	a(c)(b)
Activation (binding/association) (compound)	a(b)
Compound (activation) (phosphorylation)	c(a)(+p)
Null	n
Activation (phosphorylation) (inhibition)	a(+p)(ind)

Therefore, we have reason to believe the other seemingly irrelevant pathways also have a function in cisplatin resistance and this requires further investigation. Also, pathway analysis showed equally important roles and functions as GO analysis.

Investigating genes involved in significant pathways to form signal transduction network, 337 genes were found in common that may affect the cancer cell cisplatin resistance. Among them, CTNNB1, PLCG2 and SRC performed as the center of the network with the highest degree and ITGB8, PLCB1 and CNTNAP2 were the 3 main genes with the highest frequency. CTNNB1 encodes the core factor of Wnt signaling pathway β-catenin. An increasing number of reports have been published regarding β-catenin even Wnt/β-catenin signaling differently expressed in cisplatin-treated cancer cells (35-37). PLCG2 encodes phospholipase C which is thought to mediate Ca^{2+} signaling to alter cisplatin sensitivity in head and neck squamous cell carcinoma (38). SRC is a classic mRNA that activates the tyrosine phosphorylation of several cell pathways and was found to induce cisplatin resistance by increasing the repair of cisplatin-DNA inter-strand cross-links in human gallbladder adenocarcinoma cells early in 1999 (39). Meanwhile, we focused on the genes with the highest repetition frequency in the network. There is still no report on the role of ITGB8 in cisplatin resistance but it has been found to suppress tumor growth regulated by miRNA-93 (40). PLCB1 and CNTNAP2 mainly focus their function on neurological disease (41-44). Based on these records, the network guides our attention more on genes with higher degree but not on genes that appear more in different cell lines. Furthermore, several genes with high degree in the network have also been found to have a role in cisplatin resistance such as EGFR and PRKC. This network provides us with a number of potential genes that may relate to cancer cisplatin resistance and guide us for further investigation.

The above results suggest that differences in gene expression exist between 7 pairs of cancer cell lines. These genes encode proteins involved in different GOs and signal pathways, the disruption of which can cause cisplatin resistance. Several genes and pathways provide potential candidates for distinguishing between types of cancer and whether they contain characteristics of cisplatin resistance in the future. This distinction will aid in the diagnosis and prevention of cancer cell cisplatin resistance, based on their different characteristics.

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