Topological Network Analysis of Differentially Expressed Genes in Cancer Cells with Acquired Gefitinib Resistance

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Abstract. *Background/Aim: Despite great effort to elucidate the process of acquired gefitinib resistance (AGR) in order to develop successful chemotherapy, the precise mechanisms and genetic factors of such resistance have yet to be elucidated. Materials and Methods: We performed a cross-platform meta-analysis of three publically available microarray datasets related to cancer with AGR. For the top 100 differentially expressed genes (DEGs), we clustered functional modules of hub genes in a gene co-expression network and a protein-protein interaction network. We conducted a weighted correlation network analysis of total DEGs in microarray dataset GSE 34228. The identified DEGs were functionally enriched by Gene Ontology (GO) function and KEGG pathway. Results: We identified a total of 1,033 DEGs (510 up-regulated, 523 down-regulated, and 109 novel genes). Among the top 100 up- or down-regulated DEGs, many genes were found in different types of cancers and tumors. Through integrative analysis of two systemic networks, we selected six hub DEGs (Pre-B-cell leukemia homeobox1, Transient receptor potential cation channel subfamily C member 1, AXL receptor tyrosine kinase, S100 calcium binding protein A9, S100 calcium binding protein A8, and Nucleotide-binding oligomerization domain containing 2) associated with calcium homeostasis and signaling, apoptosis, transcriptional regulation, or chemoresistance. We confirmed a correlation of expression of these genes in the microarray dataset. Conclusion: Our study may lead to comprehensive insights into the complex mechanism of AGR and to novel gene expression signatures useful for further clinical studies.*

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Although chemotherapy is the most common treatment for various cancer types, the development of acquired resistance to anticancer drugs remains a serious problem, leading to a majority of patients who initially responded to anticancer drugs suffering the recurrence or metastasis of their cancer (1-3). Acquired drug resistance (ADR) is known to have a multi-factorial etiology, with specific associations that include ethnicity, genetic alterations, epigenetic imbalances, and environmental variations.

Gefitinib, an orally-active synthetic anilinoquinazoline, is a first-generation epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitor that was approved in 2003 by the US Food and Drug Administration for the second-line treatment of advanced non-small cell lung cancer (NSCLC) that harbors *EGFR*-activating mutations, such as an in-frame deletion in exon 19 or an L858R substitution in exon 21 (4, 5). To antagonize the tyrosine kinase activity of EGFR, gefitinib reversibly binds to the adenosine triphosphate binding pocket of the intracellular tyrosine kinase domain, blocking its autophosphorylation and resulting in the inhibition of subsequent downstream signal transduction (6). For example, inhibition of the downstream signaling pathway (such as phosphatidylinositol-3-kinase (PI3K)-Akt kinase (AKT)-mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways prevent cell proliferation, inhibition of programmed cell death (apoptosis), angiogenesis, invasion, or metastasis in many types of epithelial cancers. Despite an early positive clinical response, most patients with *EGFR*-mutant NSCLC eventually acquire resistance to gefitinib, resulting in the chemotherapeutic failures. Several possible mechanisms for acquired gefitinib resistance (AGR)

in lung cancer have been proposed, including the following: (i) alterations in the target oncogene, such as alternate expression of tyrosine kinase isoforms and second-site mutations in the tyrosine kinase domain (*e.g.* a T790, secondary *EGFR* mutation), altered EGFR trafficking, erb-b2 receptor tyrosine kinase 3 (ERBB3) activation, and expression of the ATP-binding cassette subfamily G member 2 (ABCG2) drug-efflux transporter; (ii) by passing of drug inhibition by oncogene addiction, such as the compensatory activation of downstream signaling pathways and redundant activation of other survival pathways (*e.g.* mesenchymal–epithelial transition factor amplification, activation of vascular endothelial growth factor, insulin-like growth factor-1, or integrin B1 pathways, hepatocyte growth factor overexpression, and loss of the phosphatase and tensin homolog, and (iii) histological transformation (*e.g.* epithelial–mesenchymal transformation and small-cell transformation). However, none of these mechanisms has provided a full explanation for AGR, and no definitive genetic factors have been reported for AGR in epithelial cancer, including NSCLC (7-10).

Because cancer research needs insightful observations to determine complex etiologies, researchers introduced high-throughput microarrays to investigate the expression of multiple genes under specific conditions (11, 12). In three published reports of microarray studies on AGR cancer, many differentially expressed genes (DEGs) were suggested as candidate biomarkers of AGR but these individual studies were limited by small sample sizes, low sample quality, and differences in laboratory protocols (13, 14). In order to minimize the uncertainty in these findings, we identified the DEGs that were consistently detected in paired samples from all microarray datasets, using a cross-platform meta-analysis. To organize the results, we also approached available data topologically, performing an integrative analysis of the DEGs at the gene or protein level.

To our knowledge, this is the first report of a cross-platform meta-analysis of multiple gene-expression profiles from microarray datasets associated with AGR.

Materials and Methods

Selection of microarray datasets qualified for meta-analysis. We thoroughly evaluated the suitability of microarray datasets retrieved on Gene Expression Omnibus (GEO) database of National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress database of the European Molecular Biology Laboratory–European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/arrayexpress/), according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines published in 2009 (15).

Meta-analysis of microarray datasets with different platforms. We performed meta-analysis of multiple gene-expression profiles in microarray datasets obtained using different platforms by means of rank product algorithm (RankProd package in R, http://www.rproject.org/) implemented in the INMEX online program (http://inmex.ca/INMEX/) (16, 17). Considering AGR cells derived from the same parental cell line, we arbitrarily fixed the sample pair. Before the datasets were analyzed, all probe IDs from each dataset were annotated as EntrezIDs for data consistency, and intensity values for gene expression were log2-transformed and processed by quantile normalization (limma package in R). A list of DEGs (up- or down-regulated) were identified based on p-value (where threshold was $p<0.05$) and foldchange (FC) level in a given number of replicates multiplied across different microarray datasets under the nonparametric algorithm, which is statistically rigorous but biologically intuitive.

Gene Ontology (GO) hierarchy and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. To discern the implication of DEGs in cancer cell lines with AGR, functional enrichment analysis of GO hierarchy (biological process, molecular function, and cellular component) and KEGG pathways were carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) web-accessible bioinformatics program (http://david.abcc.ncifcrf.gov/) under a significance threshold of $p<0.05$.

Gene co-expression network analysis. To construct gene co-expression networks for each of the top 100 up- or down-regulated DEGs, we imported the DEG lists into an extensive database of previously discovered networks and screened for significant gene–gene interactions using the online GeneMANIA program (http://www.genemania.org/) (18, 19). The correlation coefficient between a DEG and additional genes in its network was determined by a GO term (biological process)-based weighting and then was filtered to include only those gene co-expressions above a significance threshold of 0.05.

From the 10 up-regulated or down-regulated genes with the most connected edges in the co-expression networks, the distinct functional modules of the hub DEGs and additional related genes were identified by the fast-greedy HE (G) algorithm of the Community Clusters GLay plugin (http://cytoscape.wodaklab.org/wiki/CommunityStructureLayout) using Cytoscape software (http://www.cytoscape.org/) (20).

Protein–protein interaction (PPI) network analysis. To construct a PPI network for each list of proteins encoded by the top 100 up- or down-regulated DEGs, we imported the lists into the extensive database of already-known networks and screened significant PPIs under the Biological General Repository for Interaction Datasets (BioGRID) (http://thebiogrid.org/). In the top 10 up- or down-regulated protein list with the most connected edges in the PPI network, subnetwork between the hub DEGs-encoding proteins and additional related proteins were analyzed by using the Cytoscape plugin, ClusterONE (http://apps.cytoscape.org/apps/clusterone) (21, 22).

Weighted gene correlation network analysis (WGCNA). To construct a co-expression network of the identified total DEGs in cancer cell lines with AGR, we evaluated Pearson's correlation coefficient values across 1692 microarray probes in the normalized datasets of GSE 34228, which was analyzed using the most samples, by WGCNA (23). During the WGCNA procedure, briefly, Pearson's correlation matrices for all genes were calculated and transformed by raising all values to a power β (soft thresholding as biological networks are small-world and scale-free). Finally, gene co-expression modules were identified from the hierarchical cluster tree by using a dynamic tree cut procedure (24).

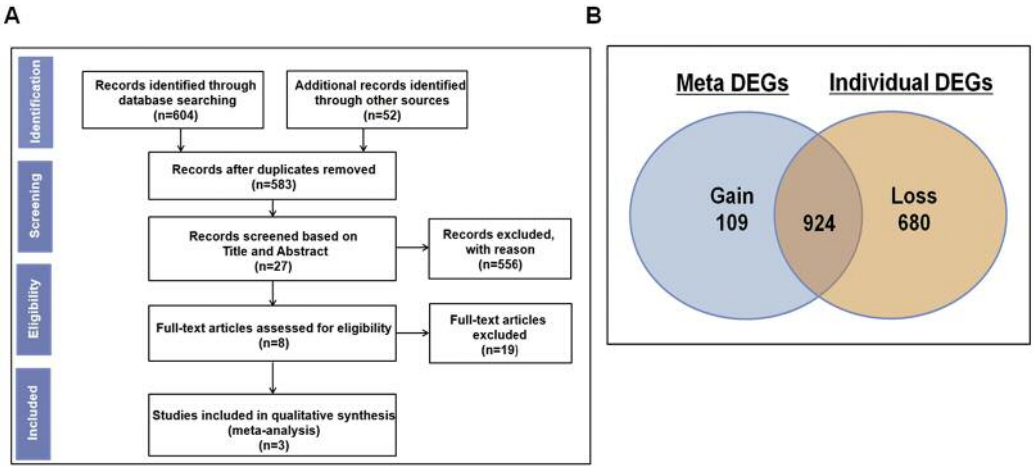

Figure 1. *Differential gene expression profiles of this meta-analysis. A: Selection of microarray datasets for meta-analysis of the acquired gefitinib resistant cancer cell lines, according to Prisma 2009 flow diagram. B: Venn diagram showing the number of significant associations between differentially expressed genes (DEGs) identified from the meta-analysis of multiple datasets (Meta-DE) and DEGs identified from the individual analysis of each dataset (individual-DE).*

Table I. *Characteristics of individual studies retrieved from Gene Expression Omnibus for meta-analysis.*

Dataset	Sample		Drug	Cancer cell	Platform
	Gef-S	Gef-R			
GSE34228	26	26	Gefitinib	Lung cancer (PC9)	Agilent-014850 Whole Human Genome Microarray 4×44K
GSE10696	3	3	Gefitinib	Epidermoid carcinoma (A431)	Affymetrix Human Genome U133 Plus 2.0 Array
GSE38302	1	3	Gefitinib	Lung cancer (PC9)	Agilent-028004 SurePrint G3 Human GE 8×60K

GSE; Gene expression series, Gef-S; gefitinib-sensitive, Gef-R; gefitinib-resistant

Results

Selection of microarray datasets for meta-analysis related to AGR. Three microarray datasets containing 62 GEO samples were extracted from the GEO database of The NCBI, which met our criteria for meta-analysis (Figure 1A). All three GEO series (GSEs) were microarray expression profiles of only the cancer cell lines that acquire drug resistance by stepwise exposure to increasing doses of gefitinib (Table I). The microarray results of three GSEs were achieved by using two cancer cell lines such as lung cancer (GSE34228 and 38302) and epidermoid carcinoma (GSE10696).

Identification of up- and down-regulated DEGs by meta-analysis. From cross-platform microarray meta-analysis, we identified total 1033 DEGs including 510 up- and 523 down-regulated genes across the three microarray datasets under the significance threshold of $p<0.05$. While 109 "gained" genes were uniquely identified as DEGs in the meta-analysis, 680 "lost" genes were identified as DEGs in any individual analysis but not in the meta-analysis (Figure 1B). The "gained" genes show relatively weak, but consistent expression profiles across all three datasets, having more reliability to be declared as novel genes. But, the "lost" genes either imply inconsistent changes in expression profiles across different datasets, or large variations by different platforms or experimental errors. The 100 most significantly up- and down-regulated DEGs with $p<1.0E{-}5$ are listed in Tables II and III, respectively. In the up-regulated DEGs, genes with the largest mean $\log_2$FC were family with sequence similarity 9, member B (*FAM9B*), followed by patatin-like phospholipase domain containing 4 (*PNPLA4*) and ankyrin repeat domain 30B pseudogene 2 (*ANKRD30BP2*). The down-regulated genes with the largest mean $\log_2$FC were by descending order: *GPC6, S100A8*, and *SYNPO2L*.

A subset of top 25 dysregulated DEGs across the three microarray datasets was also visualized by heat maps showing differential expression of individual datasets (Figure 2).

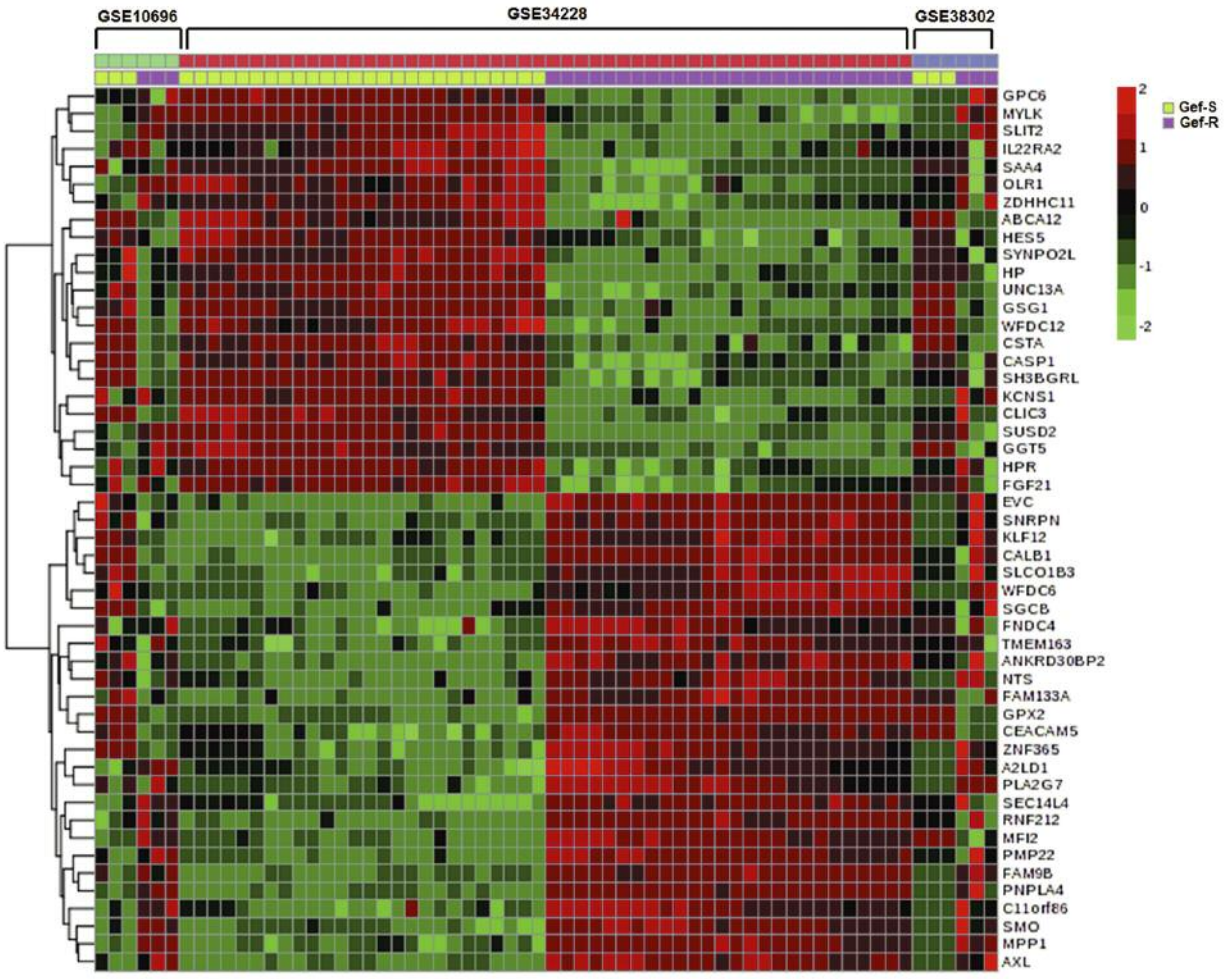

Figure 2. *Heat-map representation of expression profiles for the top 25 up- and 25 down-regulated DEGs across three datasets. Clustering of selected genes on the heat-map was performed by hierarchical clustering algorithm using average linkage method and Euclidean distance measure. Gef-S: gefitinib-senstive; Gef-R: gefitinib-resistant.*

GO hierarchy and KEGG pathway enrichment analysis of total DEGs. Considering all 1,033 DEGs obtained by the meta-analysis, the most over-represented GO terms in biological processes were enriched in the following descending order: "inflammatory response", "epidermis development", and "response to inorganic substance" (Table IV). The most enriched GO terms in molecular function and cellular component were "calcium ion binding" and "plasma membrane", respectively. The most enriched KEGG pathway terms were as follows (in descending order): "calcium signaling pathway", "phosphatidylinositol signaling system", "cytokine–cytokine receptor interaction", and "pathways in cancer".

Gene co-expression network analysis of DEGs. To interpret the biological meaning of the identified DEGs at the gene level, we constructed a co-expression network for the top 100 up- and down-regulated DEGs with significant interaction relation composed of 144 nodes/446 edges and 124 nodes/550 edges, respectively. From the co-expression network of up-regulated DEGs, the list of top 10 hub genes was determined in order of number of the interacting edges as follows (in order): peripheral myelin protein 22 (*PMP22*), echinoderm microtubule associated protein like 1 (*EML1*), secretogranin II (*SCG2*), sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1 (*SPOCK1*), pre-B-cell leukemia homeobox 1 (*PBX1*), transient receptor potential cation channel, subfamily C, member 1 (*TRPC1*), growth arrest-specific 6 (*GAS6*), phosphodiesterase 2A, cGMP-stimulated (*PDE2A*), AXL receptor tyrosine kinase (*AXL*), and prostaglandin I2 synthase (*PTGIS*). In the network of down-regulated DEGs, the top 10 hub genes with the most connected edges were peptidase inhibitor 3, skin-derived (*PI3*), S100 calcium binding protein A9 (*S100A9*), S100A8, chemokine

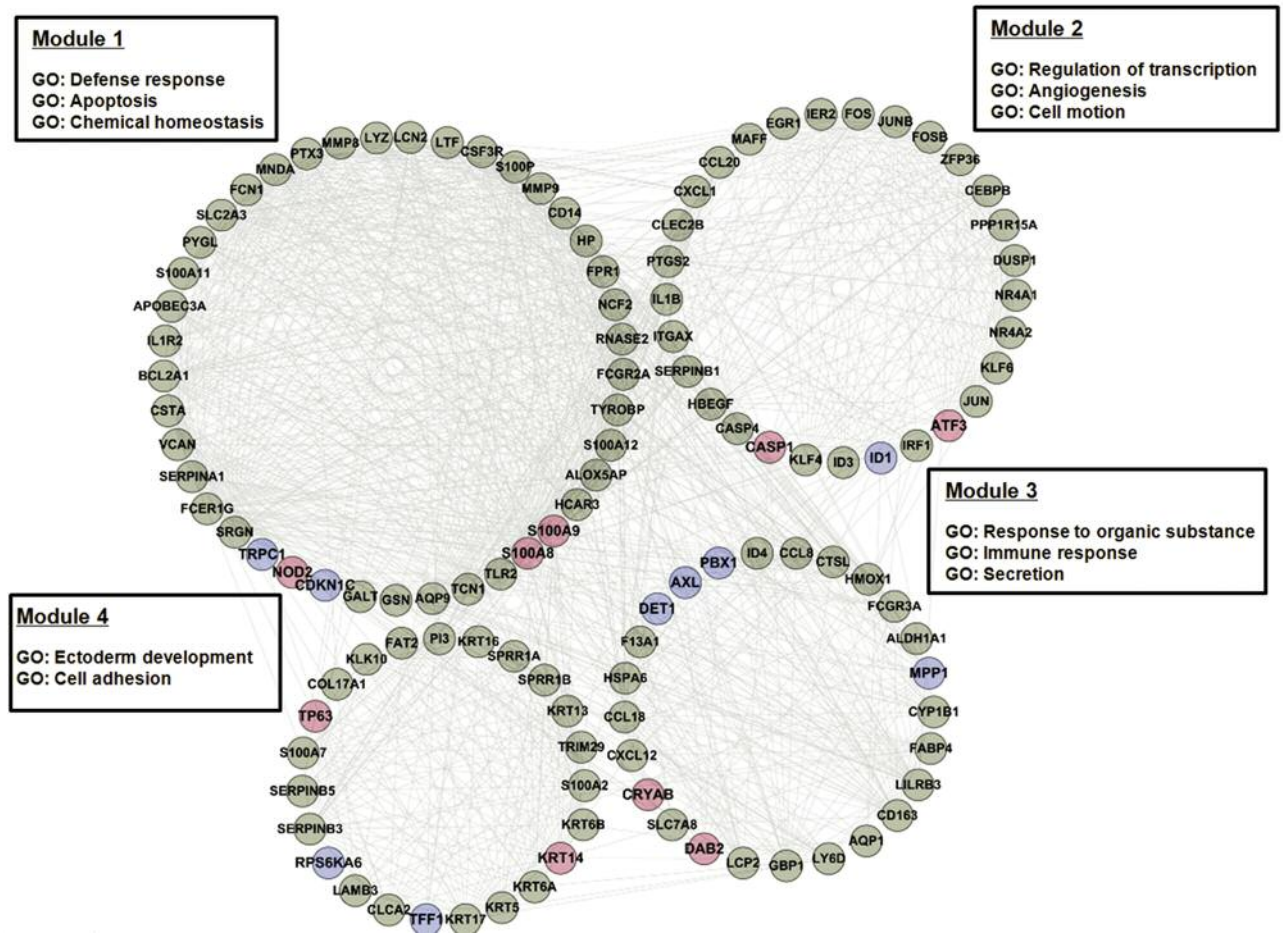

Figure 3. *Functional modules of hub genes in gene co-expression network. From gene co-expression networks of the top 100 up- or down-regulated DEGs, four functional modules were clustered by each of the top 10 most interacting hub genes of up- or down-regulated DEGs. The color of node signifies the following: Blue: up-regulated DEGs, Red: down-regulated DEGs, and Light brown: additional genes from GeneMANIA program.*

(C-X-C motif) ligand 1 (*CXCL1*), cystatin A (*CSTA*), PDZK1 interacting protein 1 (*PDZK1IP1*), serpin peptidase inhibitor, clade B (ovalbumin), member 2 (*SERPINB2*), nucleotide-binding oligomerization domain containing 2 (*NOD2*), serum amyloid A4, constitutive (*SAA4*), and chitinase 3-like 2 (*CHI3L2*).

The distinct modules of the hub DEGs and their interacting genes were further identified by fast-greedy HE (G) algorithm of GLay Cytoscape plugin (Figure 3). Among the modules, "Module 1", which has the largest size formed by 41 nodes, was significantly enriched by biological process terms such as "defense response", "apoptosis", and "chemical homeostasis" of GO hierarchy.

PPI network analysis of DEGs. In order to interpret the biological meaning of the identified DEGs at the protein level, we constructed a PPI network for the proteins encoded by the top 100 up- or down-regulated DEGs, which were made by significant interaction including 196 nodes/237 edges and 438 nodes/500 edges respectively.

From the PPI network of proteins encoded by up-regulated DEGs, the list of the top 10 hub proteins was determined in order of number of the interacting edges as follows (in order): ribosomal protein S6 kinase, polypeptide 6 (RPS6KA6), PBX1, inhibitor of DNA binding 1 (ID1), TRPC1, small nuclear ribonucleoprotein polypeptide N (SNRPN), AXL, cyclin-dependent kinase inhibitor 1C (CDKN1C), trefoil factor 1 (TFF1), membrane protein, palmitoylated 1 (MPP1), and de-etiolated homolog 1 (DET1) (Figure 4).

In the same manner, the top 10 hub proteins encoded by down-regulated DEGs were tumor protein p63 (TP63), crystallin, alpha B (CRYAB), S100A9, activating transcription factor 3 (ATF3), disabled homolog 2 (DAB2), histone cluster 1, H1a (HIST1H1A), S100A8, keratin 14 (KRT14), NOD2, and caspase 1 (CASP1).

Table II. *The top 100 most significantly up-regulated genes in the meta-analysis.*

Entrez ID	Gene symbol	Log2 FC	Gene name
171483	*FAM9B*	−3.90125	Family with sequence similarity 9, member B
8228	*PNPLA4*	−3.39019	Patatin-like phospholipase domain containing 4
149992	*ANKRD30BP2*	−3.36623	Ankyrin repeat domain 30B pseudogene 2
285498	*RNF212*	−3.26040	Ring finger protein 212
4354	*MPP1*	−3.24646	Membrane protein, palmitoylated 1, 55 kDa
87769	*A2LD1*	−3.04257	Gamma-glutamylamine cyclotransferase
793	*CALB1*	−2.83337	Calbindin 1, 28kDa
558	*AXL*	−2.71814	AXL receptor tyrosine kinase
2121	*EVC*	−2.69040	Ellis van Creveld syndrome
5376	*PMP22*	−2.68507	Peripheral myelin protein 22
6608	*SMO*	−2.65570	Smoothened, frizzled class receptor
151827	*LRRC34*	−2.65214	Leucine rich repeat containing 34
254439	*C11orf86*	−2.64451	Chromosome 11 open reading frame 86
7941	*PLA2G7*	−2.57645	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
11278	*KLF12*	−2.56561	Kruppel-like factor 12
28234	*SLCO1B3*	−2.56060	Solute carrier organic anion transporter family, member 1B3
84171	*LOXL4*	−2.52231	Lysyl oxidase-like 4
4241	*MFI2*	−2.51589	Antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96
6695	*SPOCK1*	−2.50976	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1
64838	*FNDC4*	−2.50655	Fibronectin type III domain containing 4
284904	*SEC14L4*	−2.49981	SEC14-like 4
6638	*SNRPN*	−2.45761	Small nuclear ribonucleoprotein polypeptide N
22891	*ZNF365*	−2.45694	Zinc finger protein 365
94031	*HTRA3*	−2.43569	HtrA serine peptidase 3
399818	*METTL10*	−2.41988	Methyltransferase like 10
6443	*SGCB*	−2.39483	Sarcoglycan, beta
89853	*FAM125B*	−2.37603	Family with sequence similarity 125, member B
140870	*WFDC6*	−2.34668	WAP four-disulfide core domain 6; serine peptidase inhibitor-like
57732	*ZFYVE28*	−2.33267	Zinc finger, FYVE domain containing 28
81615	*TMEM163*	−2.32293	Transmembrane protein 163
5624	*PROC*	−2.31267	Protein C
148808	*MFSD4*	−2.28194	Major facilitator superfamily domain containing 4
4922	*NTS*	−2.25746	Neurotensin
201456	*FBXO15*	−2.23269	F-Box protein 15
7136	*TNNI2*	−2.22495	Troponin I type 2
285220	*EPHA6*	−2.22490	EPH receptor A6
286499	*FAM133A*	−2.21997	Family with sequence similarity 133, member A
2877	*GPX2*	−2.20498	Glutathione peroxidase 2
2621	*GAS6*	−2.20071	Growth arrest-specific 6
116154	*PHACTR3*	−2.16645	Phosphatase and actin regulator 3
4320	*MMP11*	−2.14391	Matrix metallopeptidase 11
2568	*GABRP*	−2.09987	Gamma-aminobutyric acid A receptor
23059	*CLUAP1*	−2.08400	Clusterin associated protein 1
400655	*LOC400655*	−2.08052	Hypothetical gene supported by BC013370; BC034583
79923	*NANOG*	−2.07658	Nanog homeobox pseudogene 8
23671	*TMEFF2*	−2.06106	Transmembrane protein with EGF-like and two follistatin-like domains 2
8622	*PDE8B*	−2.05822	Phosphodiesterase 8B
137392	*FAM92A1*	−2.03892	Family with sequence similarity 92, member A1
646113	*FLJ43390*	−2.03626	Hypothetical LOC646113
2562	*GABRB3*	−2.03076	Gamma-aminobutyric acid A receptor, beta 3
171484	*FAM9C*	−2.00399	Family with sequence similarity 9, member C
80712	*ESX1*	−2.00289	ESX homeobox 1
80235	*PIGZ*	−1.99490	Phosphatidylinositol glycan anchor biosynthesis, class Z
5138	*PDE2A*	−1.97341	Phosphodiesterase 2A, cGMP-stimulated
1048	*CEACAM5*	−1.96272	Carcinoembryonic antigen-related cell adhesion molecule 5
9863	*MAGI2*	−1.95602	Membrane associated guanylate kinase
84952	*CGNL1*	−1.94479	Cingulin-like 1
8187	*ZNF239*	−1.93698	Zinc finger protein 239

Table II. *Continued*

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Entrez ID	Gene symbol	Log2 FC	Gene name
8784	*TNFRSF18*	−1.92713	Tumor necrosis factor receptor superfamily, member 18
2054	*STX2*	−1.91054	Syntaxin 2
338707	*B4GALNT4*	−1.90519	Beta-1,4-N-acetyl-galactosaminyl transferase 4
200407	*CREG2*	−1.90473	Cellular repressor of E1A-stimulated genes 2
114879	*OSBPL5*	−1.90453	Oxysterol binding protein-like 5
27330	*RPS6KA6*	−1.89919	Ribosomal protein S6 kinase, 90 kDa, polypeptide 6
10125	*RASGRP1*	−1.89060	RAS guanyl releasing protein 1
27092	*CACNG4*	−1.88590	Calcium channel, voltage-dependent, gamma subunit 4
148641	*SLC35F3*	−1.88486	Solute carrier family 35, member F3
7857	*SCG2*	−1.87220	Secretogranin II
7220	*TRPC1*	−1.87136	Transient receptor potential cation channel, subfamily C, member 1
1028	*CDKN1C*	−1.87109	Cyclin-dependent kinase inhibitor 1C
138311	*FAM69B*	−1.86758	Family with sequence similarity 69, member B
55283	*MCOLN3*	−1.85946	Mucolipin 3
26049	*FAM169A*	−1.85476	Family with sequence similarity 169, member A
5141	*PDE4A*	−1.84987	Phosphodiesterase 4A, cAMP-specific
59285	*CACNG6*	−1.84673	Calcium channel, voltage-dependent, gamma subunit 6
57007	*CXCR7*	−1.84200	Chemokine (C-X-C motif) receptor 7
5542	*PRB1*	−1.81966	Proline-rich protein BstNI subfamily 1
5740	*PTGIS*	−1.81753	Prostaglandin I2 (prostacyclin) synthase
7504	*XK*	−1.80963	X-linked Kx blood group
120376	*C11orf93*	−1.78835	Uncharacterized protein LOC120376
9966	*TNFSF15*	−1.78234	Tumor necrosis factor (ligand) superfamily, member 15
7031	*TFF1*	−1.78168	Trefoil factor 1
65009	*NDRG4*	−1.77721	NDRG family member 4
58473	*PLEKHB1*	−1.77451	Pleckstrin homology domain containing
137209	*ZNF572*	−1.76607	Zinc finger protein 572
399693	*MGC50722*	−1.76511	Hypothetical MGC50722
26960	*NBEA*	−1.76316	Neurobeachin
148418	*SAMD13*	−1.76037	Sterile alpha motif domain containing 13
2530	*FUT8*	−1.75395	Fucosyltransferase 8
4756	*NEO1*	−1.73991	Neogenin homolog 1
3397	*ID1*	−1.73948	Inhibitor of DNA binding 1
2009	*EML1*	−1.73573	Echinoderm microtubule associated protein like 1
57119	*SPINLW1*	−1.73023	Serine peptidase inhibitor-like, with Kunitz and WAP domains 1
2039	*EPB49*	−1.73018	Erythrocyte membrane protein band 4.9
57547	*ZNF624*	−1.73016	Zinc finger protein 624
57493	*HEG1*	−1.72849	HEG homolog 1
29785	*CYP2S1*	−1.72804	Cytochrome P450, family 2, subfamily S, polypeptide 1
55070	*DET1*	−1.71702	De-etiolated homolog 1
5087	*PBX1*	−1.71510	Pre-B-cell leukemia homeobox 1
9249	*DHRS3*	−1.71204	Dehydrogenase/reductase member 3

Log_2FC=log2 (class1/class2), FC; fold change, class1; gefitinib-sensitive, class2; gefitinib-resistant.

The 15 module clusters including the hub proteins were further identified by density of nodes and *p*-value of ClusterONE Cytoscape plugin and six of these clusters shared common genes with the top 10 hub gene lists of the gene co-expression network (Figure 5).

WGCNA of the identified total DEGs in cancer cell lines with AGR. To investigate the functional module eigen (ME) of genes that were highly correlated in microarray dataset GSE34228 with the most samples, we conducted the WGCNA of total DEGs by R package WGCNA under a scale-free topology of the power adjacency function parameter of 11.

The closely-interacting genes within the network were subdivided into eight distinct MEs using the dynamic hybrid tree-cutting algorithm and were identified by functional enrichment of GO hierarchy and KEGG pathway (Figure 6). Among them, “MEgrey” was the largest ME of 405 nodes and was significantly enriched by GO terms “regulation of cell proliferation” and KEGG terms “cytokine–cytokine receptor interaction”.

Table III. *The top 100 most significantly down-regulated genes in the meta-analysis.*

Entrez ID	Gene symbol	Log2 FC	Gene name
10082	*GPC6*	4.872629	Glypican 6
6279	*S100A8*	3.831239	S100 calcium binding protein A8
79933	*SYNPO2L*	3.568912	Synaptopodin 2-like
26154	*ABCA12*	3.490312	ATP-binding cassette, sub-family A (ABC1), member 12
1475	*CSTA*	3.397182	Cystatin A (stefin A)
128488	*WFDC12*	3.301753	WAP four-disulfide core domain 12
3787	*KCNS1*	3.250585	Potassium voltage-gated channel, modifier subfamily S, member 1
6451	*SH3BGRL*	3.218268	SH3 domain binding glutamate-rich protein like
3250	*HPR*	3.180568	Haptoglobin-related protein
834	*CASP1*	3.148989	Caspase 1, apoptosis-related cysteine peptidase
83445	*GSG1*	3.123732	Germ cell associated 1
388585	*HES5*	3.107186	Hes family bHLH transcription factor 5
3240	*HP*	3.074056	Haptoglobin
6699	*SPRR1B*	3.049195	Small proline-rich protein 1B
5266	*PI3*	3.045197	Peptidase inhibitor 3, skin-derived
149708	*WFDC5*	3.008763	WAP four-disulfide core domain 5
56241	*SUSD2*	2.984918	Sushi domain containing 2
51806	*CALML5*	2.977824	Calmodulin-like 5
23025	*UNC13A*	2.862026	Unc-13 homolog A (C. elegans)
6291	*SAA4*	2.749179	Serum amyloid A4, constitutive
4689	*NCF4*	2.69263	Neutrophil cytosolic factor 4
9022	*CLIC3*	2.67059	Chloride intracellular channel 3
5055	*SERPINB2*	2.63490	Serpin peptidase inhibitor, clade B (ovalbumin), member 2
10158	*PDZK1IP1*	2.60480	PDZK1 interacting protein 1
3576	*IL8*	2.59001	Interleukin 8
3854	*KRT6B*	2.58998	Keratin 6B
216	*ALDH1A1*	2.58969	Aldehyde dehydrogenase 1 family, member A1
9353	*SLIT2*	2.57949	Slit homolog 2
26291	*FGF21*	2.54931	Fibroblast growth factor 21
1E+08	*LOC100130476*	2.52279	Similar to hCG2036711
3024	*HIST1H1A*	2.51167	Histone cluster 1, H1a
4638	*MYLK*	2.50508	Myosin light chain kinase
2687	*GGT5*	2.50050	Gamma-glutamyltransferase 5
57110	*HRASLS*	2.47448	HRAS-like suppressor
765	*CA6*	2.46709	Carbonic anhydrase VI
117286	*CIB3*	2.42847	Calcium and integrin binding family member 3
6278	*S100A7*	2.40512	S100 calcium binding protein A7
81553	*FAM49A*	2.39138	Family with sequence similarity 49, member A
3598	*IL13RA2*	2.37391	Interleukin 13 receptor, alpha 2
79844	*ZDHHC11*	2.37258	Zinc finger, DHHC-type containing 11
3853	*KRT6A*	2.36484	Keratin 6A
116379	*IL22RA2*	2.35407	Interleukin 22 receptor, alpha 2
11254	*SLC6A14*	2.31839	Solute carrier family 6 (amino acid transporter), member 14
1580	*CYP4B1*	2.30726	Cytochrome P450, family 4, subfamily B, polypeptide 1
2981	*GUCA2B*	2.29064	Guanylate cyclase activator 2B
80736	*SLC44A4*	2.28010	Solute carrier family 44, member 4
1117	*CHI3L2*	2.24191	Chitinase 3-like 2
1410	*CRYAB*	2.23727	Crystallin, alpha B
6702	*SPRR2C*	2.22302	Small proline-rich protein 2C (pseudogene)
24	*ABCA4*	2.22258	ATP-binding cassette, sub-family A (ABC1), member 4
8424	*BBOX1*	2.18805	Butyrobetaine (gamma), 2-oxoglutarate dioxygenase 1
1303	*COL12A1*	2.17887	Collagen, type XII, alpha 1
4973	*OLR1*	2.14220	Oxidized low density lipoprotein (lectin-like) receptor 1
64881	*PCDH20*	2.12045	Protocadherin 20
2702	*GJA5*	2.07773	Gap junction protein, alpha 5, 40 kDa
10561	*IFI44*	2.06929	Interferon-induced protein 44
6280	*S100A9*	2.05150	S100 calcium binding protein A9
54550	*NECAB2*	2.03725	N-Terminal EF-hand calcium binding protein 2

Table III. *Continued*

Table III. *Continued*

Entrez ID	Gene symbol	Log2 FC	Gene name
64333	*ARHGAP9*	2.03643	Rho GTPase activating protein 9
57115	*PGLYRP4*	2.03448	Peptidoglycan recognition protein 4
733	*C8G*	2.03239	Complement component 8, gamma polypeptide
3861	*KRT14*	2.03208	Keratin 14
57514	*ARHGAP31*	2.00670	Cdc42 GTPase-activating protein
59272	*ACE2*	2.00009	Angiotensin I converting enzyme 2
684	*BST2*	1.99884	Bone marrow stromal cell antigen 2
10409	*BASP1*	1.99818	Brain abundant, membrane attached signal protein 1
1601	*DAB2*	1.99402	Disabled homolog 2
56911	*C21orf7*	1.98239	Chromosome 21 open reading frame 7
79153	*GDPD3*	1.97767	Glycerophosphodiester phosphodiesterase domain containing 3
400926	*FLJ90680*	1.96695	FLJ90680 protein
6317	*SERPINB3*	1.94860	Serpin peptidase inhibitor, clade B, member 3
8626	*TP63*	1.93193	Tumor protein p63
51702	*PADI3*	1.92478	Peptidyl arginine deiminase, type III
6406	*SEMG1*	1.90862	Semenogelin I
64127	*NOD2*	1.89057	Nucleotide-binding oligomerization domain containing 2
23569	*PADI4*	1.88711	Peptidyl arginine deiminase, type IV
84891	*ZSCAN10*	1.88550	Zinc finger and SCAN domain containing 10
4633	*MYL2*	1.88162	Myosin, light chain 2, regulatory, cardiac, slow
467	*ATF3*	1.87826	Activating transcription factor 3
374454	*KRT77*	1.87719	Keratin 77
26471	*NUPR1*	1.87228	Nuclear protein, transcriptional regulator, 1
92241	*RCSD1*	1.87127	RCSD domain containing 1
387509	*GPR153*	1.84401	G Protein-coupled receptor 153
79883	*PODNL1*	1.83313	Podocan-like 1
115362	*GBP5*	1.83305	Guanylate binding protein 5
6543	*SLC8A2*	1.83276	Solute carrier family 8, member 2
725	*C4BPB*	1.83044	Complement component 4 binding protein, beta
115572	*FAM46B*	1.82541	Family with sequence similarity 46, member B
2919	*CXCL1*	1.81746	Chemokine (C-X-C motif) ligand 1
360	*AQP3*	1.81135	Aquaporin 3
54626	*HES2*	1.80263	Hairy and enhancer of split 2
137994	*LETM2*	1.79409	Leucine zipper-EF-hand containing transmembrane protein 2
7439	*BEST1*	1.78287	Bestrophin 1
8909	*ENDOU*	1.77794	26 serine protease
440356	*LOC440356*	1.77779	Hypothetical LOC440356
55064	*C9orf68*	1.77607	Chromosome 9 open reading frame 68
57451	*ODZ2*	1.77390	Odz, odd Oz/ten-m homolog 2
126520	*PLK5*	1.77288	Polo-like kinase 5 pseudogene
118611	*C10orf90*	1.77055	Chromosome 10 open reading frame 90
2549	*GAB1*	1.76527	GRB2-associated binding protein 1

Log_2FC=log2 (class1/class2), FC; fold change, class1; gefitinib-sensitive, class2; gefitinib-resistant.

Discussion

Cancer cells that develop resistance to gefitinib are a major challenge to successful treatment and long-term survival. To date, no clear mechanism for AGR has been demonstrated. A multifarious approach to analyzing the expression patterns of multiple genes in AGR cancer cells could allow us to characterize the overall response of resistant cells and to understand the complex mechanisms underlying gefitinib resistance.

In the gene-expression patterns obtained by the meta-analysis, primary study for *p*-value and log_2FC of DEGs showed that not few of the top 100 up- or down-regulated DEGs were found in developmental processes of many types of tumor. In the case of top 20 DEGs, in particular, previous studies reported that most of the genes were involved in carcinogenesis of various types of cancer such as of the breast, prostate, stomach, ovary, and lung. In addition, some of these DEGs have been identified in cancer cells with anticancer drug resistance other than AGR, including resistance to cisplatin,

Table IV. *The top 15 Gene Ontology hierarchy and Kyoto Encyclopedia of Genes and Genomes pathway enrichment.*

ID	Term	Number of genes	*p*-Value
GO hierarchy			
GO_MF:0005509	Calcium ion binding	91	5.77E-09
GO_BP:0006954	Inflammatory response	35	9.71E-05
GO_BP:0008544	Epidermis development	23	2.59E-04
GO_MF:0008083	Growth factor activity	21	3.19E-04
GO_BP:0010035	Response to inorganic substance	24	4.79E-04
GO_BP:0043067	Regulation of programmed cell death	63	1.76E-03
GO_BP:0001525	Angiogenesis	18	1.95E-03
GO_CC:0005886	Plasma membrane	223	5.28E-03
GO_BP:0007242	Intracellular signaling cascade	87	5.81E-03
GO_BP:0000122	Negative regulation of transcription from RNA polymerase II promoter	25	6.81E-03
GO_BP:0007267	Cell-cell signaling	46	9.95E-03
GO_BP:0051050	Positive regulation of transport	21	1.36E-02
GO_MF:0005543	Phospholipid binding	16	4.80E-02
GO_CC:0005856	Cytoskeleton	81	6.31E-02
GO_CC:0031090	Organelle membrane	48	7.50E-02
KEGG pathway			
Hsa:04020	Calcium signaling pathway	20	2.48E-03
Hsa:04070	Phosphatidylinositol signaling system	11	5.59E-03
Hsa:04060	Cytokine-cytokine receptor interaction	24	1.23E-02
Hsa:05200	Pathways in cancer	28	1.53E-02
Hsa:05219	Bladder cancer	7	2.29E-02
Hsa:04666	Fc gamma R-mediated phagocytosis	10	6.62E-02
Hsa:05223	Non-small cell lung cancer	7	6.66E-02
Hsa:04115	p53 signaling pathway	8	6.96E-02
Hsa:04012	ERBB signaling pathway	9	9.22E-02
Hsa:04630	JAK-STAT signaling pathway	13	1.05E-01
Hsa:04150	mTOR signaling pathway	6	1.13E-01
Hsa:05217	Basal cell carcinoma	6	1.29E-01
Hsa:04010	MAPK signaling pathway	19	1.74E-01
Hsa:04621	NOD-like receptor signaling pathway	6	1.86E-01
Hsa:04370	VEGF signaling pathway	6	1.93E-01

trastuzumab, fluorouracil, taxane, and erlotinib. DEGs implicated include membrane protein, palmitoylated 1 (*MPP1*), *AXL, PMP22*, smoothened, frizzled class receptor (*SMO*), *S100A8*, Hes family bHLH transcription factor 5 (*HES5*), ATP-binding cassette, sub-family A, member 12 (*ABCA12*), haptoglobin (*HP*), and *PI3*. Interestingly, we identified 109 "gained" DEGs in this meta-analysis which were not discovered in the prior individual analyses. This large number of "gained" DEGs implies that our approach has a higher likelihood of identifying novel biomarkers for AGR. For the identified DEGs overall, a functional enrichment analysis revealed that a large proportion of these genes were classified as having functions related to cellular processes that occur during oncogenesis, including homeostasis, development, apoptosis, immune response, angiogenesis, and signal transduction. In our gene coexpression network, four distinct modules composed of hub DEGs were classified into typical cellular processes of AGR, including indefinite cell proliferation and anti-apoptosis (module 1), malfunctioning membrane transport of small molecules (modules 1, 3, and 4), response to chemical compounds (modules 1 and 3), abnormal cell development (modules 2 and 4), angiogenesis (module 2), and dysregulated transcription (module 2). In parallel with the gene co-expression network, 15 module clusters surrounding hub proteins were identified and enriched in the PPI network. By comparing these networks, we recognized six genes in common, including three up-regulated genes (*AXL, PBX1*, and *TRPC1*) and three down-regulated genes (*S100A9, S100A8,* and *NOD2*). It is notable that *TRPC1* and another three down-regulated genes were affiliated with "module 1" in the gene co-expression network. The DEGs in "module 1" were enriched with GO (biological process) terms relevant to calcium homeostasis and signaling or apoptosis, as observed in the hub protein clusters from the PPI network. Moreover, "calcium ion binding" and "calcium signaling pathway" ranked first in the top 15 terms of the GO hierarchy and

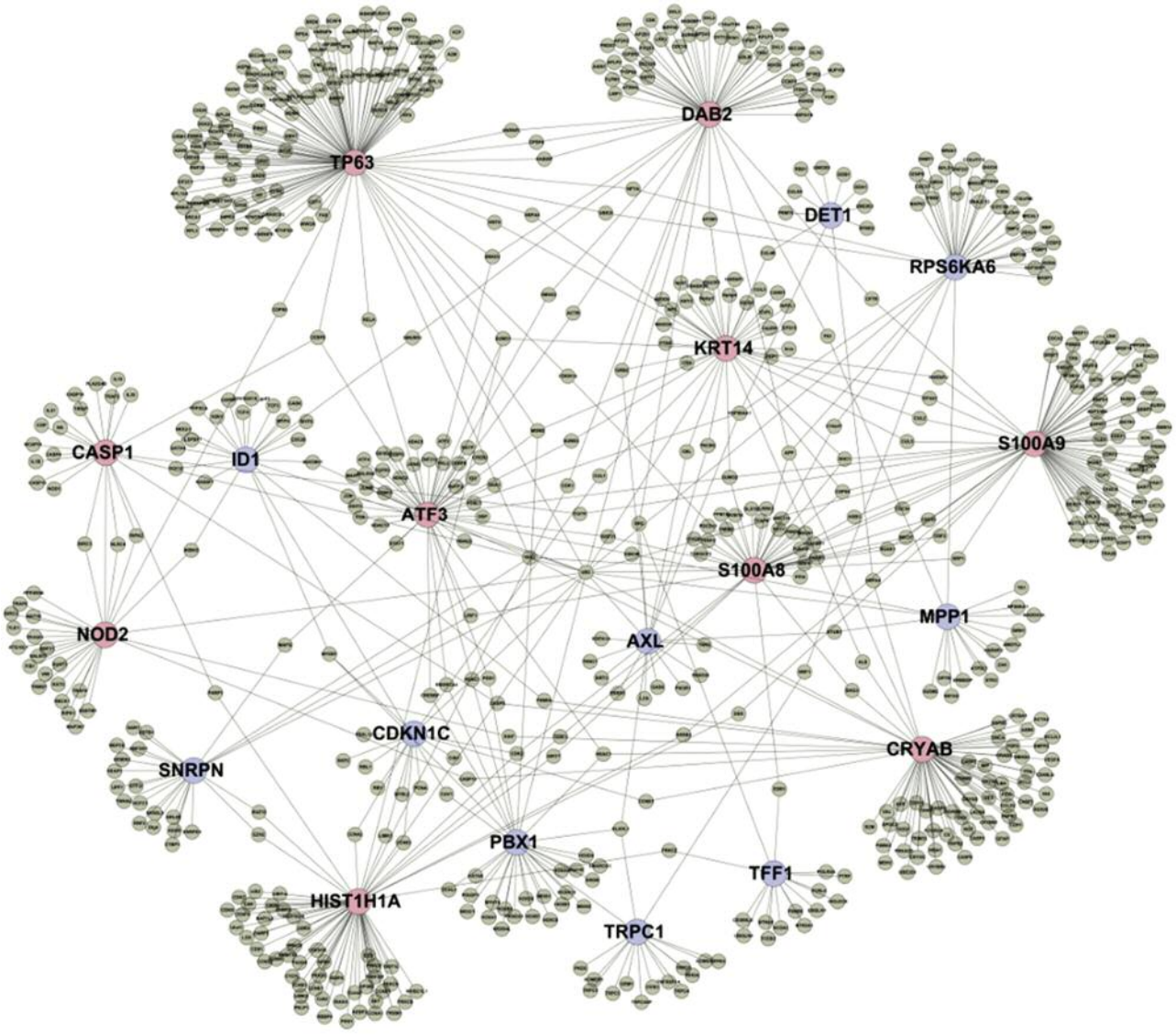

Figure 4. *Protein–protein interaction (PPI) network of top 10 lists of up- and down-regulated DEGs based on the number of interactions. We constructed the PPI network of proteins encoded by the top 100 up- or down-regulated DEGs under BioGRID program. From the PPI network, each top 10 lists of up- or down-regulated proteins were identified by the number of connected nodes and analyzed on genome-free scale in Cytoscape software. The color of node signifies proteins that are encoded by the following: Light blue: up-regulated DEGs, Light red: down-regulated DEGs, and Light brown: additional genes in BioGRID.*

KEGG pathway enrichment of DEGs overall. Many studies have suggested that dysregulated calcium homeostasis and altered calcium signaling play important roles in anti-apoptosis, tumor vascularization, invasion, and metastasis in the carcinogenesis of many tumorigenic cells (25, 26). Joshua *et al*. reported that sustained potentiation of purinergic intercellular calcium signaling was observed following transient exposure to EGF, and that this was not blocked by gefitinib or erlotinib in human glioma cells, suggesting new therapeutic approaches to gliomas and other tumors (27). The TRP ion channel family has been implicated in the regulation of cancer progression and aggressiveness by modulating calcium influx and downstream signaling in many types of cancers (28). In NSCLC cells, EGF-induced calcium entry through TRPC1 promoted cancer cell proliferation by activating EGFR and escaping G_0/G_1 cell-cycle arrest (29). S100A8 and S100A9 (a multigene family of non-ubiquitous cytoplasmic calcium-binding proteins of the EF-hand type) are

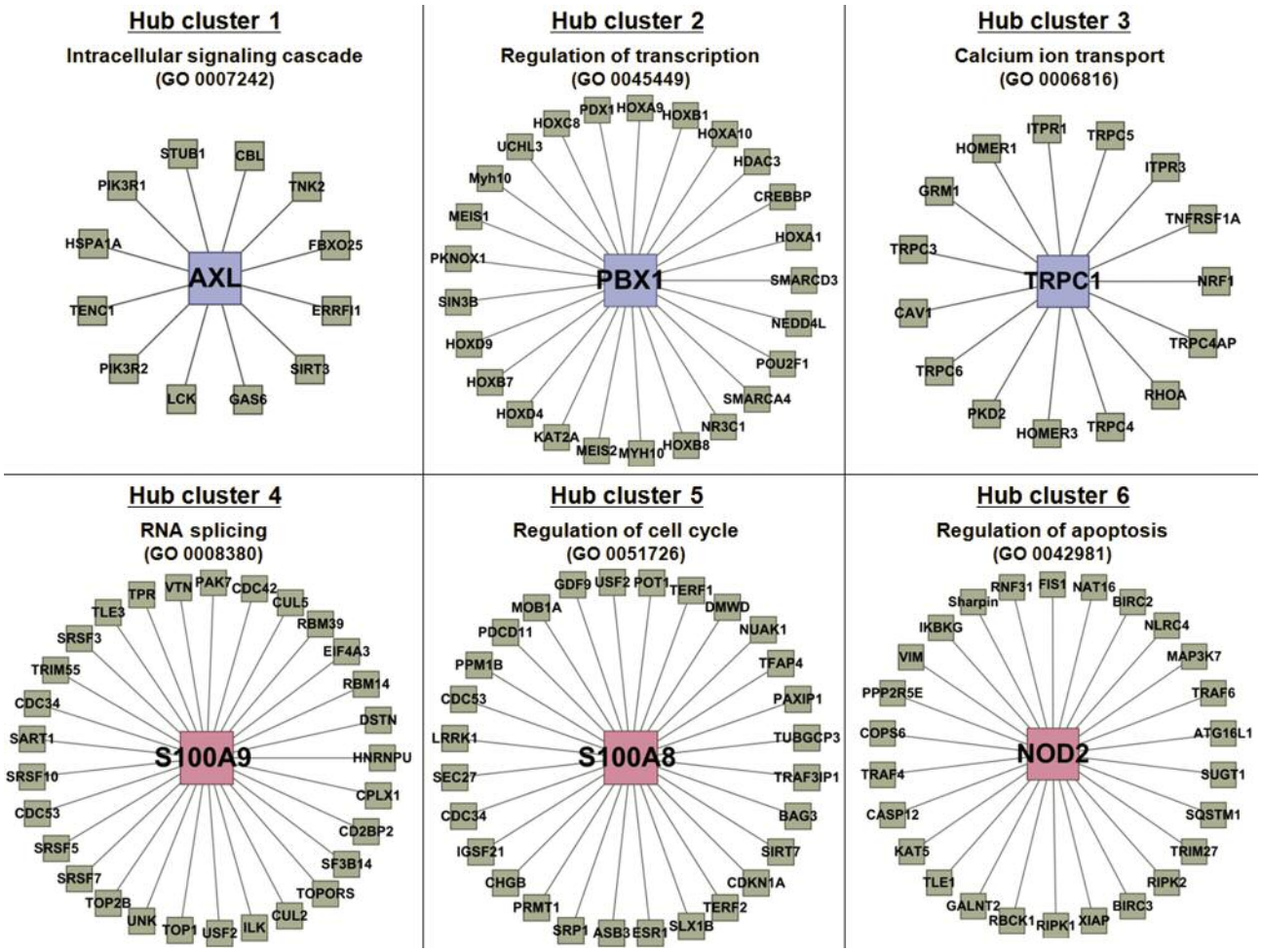

Figure 5. *Functional clusters of hub DEG-encoding proteins in PPI network. From PPI networks of proteins encoded by the top 100 up- or down-regulated DEGs, we identified functional module clusters of six hub proteins that were identical with the hub DEGs of the gene co-expression network. The color of node signifies proteins that are encoded by the following: Light blue: up-regulated DEGs, Light red: down-regulated DEGs, and Light brown: additional genes in BioGRID program.*

involved in tumor development or progression and have been recently revealed to be associated with the progression of carcinoma cells *via* the Wnt/β-catenin pathway or in MMP2 expression (30-32). NOD2 is known to induce chronic inflammation by mediating anti-apoptosis and autophagy pathways in cancer development (33). AXL and PBX1, both found in "module 2" of the gene co-expression network, have also been reported to be involved in carcinogenesis and tumor development. For example, the AXL receptor tyrosine kinase participates in cell proliferation, invasion and metastasis, and chemoresistance in many types of solid cancers, and causes acquired resistance to EGFR-tyrosine kinase inhibitors by mediating the EMT pathway in NSCLC cells (34). PBX1 forms heterodimeric transcription complexes with other homeodomain-containing nuclear proteins, such as HOX and HEIS, and regulates expression of important genes in organogenesis, hematopoiesis, and tumorigenesis (35). To determine the correlation of the six candidate DEGs from gene expression profiles of the microarray dataset, we performed a WGCNA of all identified DEGs in GSE34228 and identified eight functional MEs in which genes with highly correlated co-expression were grouped by biological terms of carcinogenesis through functional enrichment. Through our analyses, we confirmed that five DEGs clustered within MEgrey, with NOD2 included in MEred, are contiguous with each other in a hierarchical clustering tree of MEs.

In conclusion, the present study provided comprehensive insights into the complex nature and mechanisms of AGR and novel gene-expression signatures that may be useful for clinical chemotherapy studies.

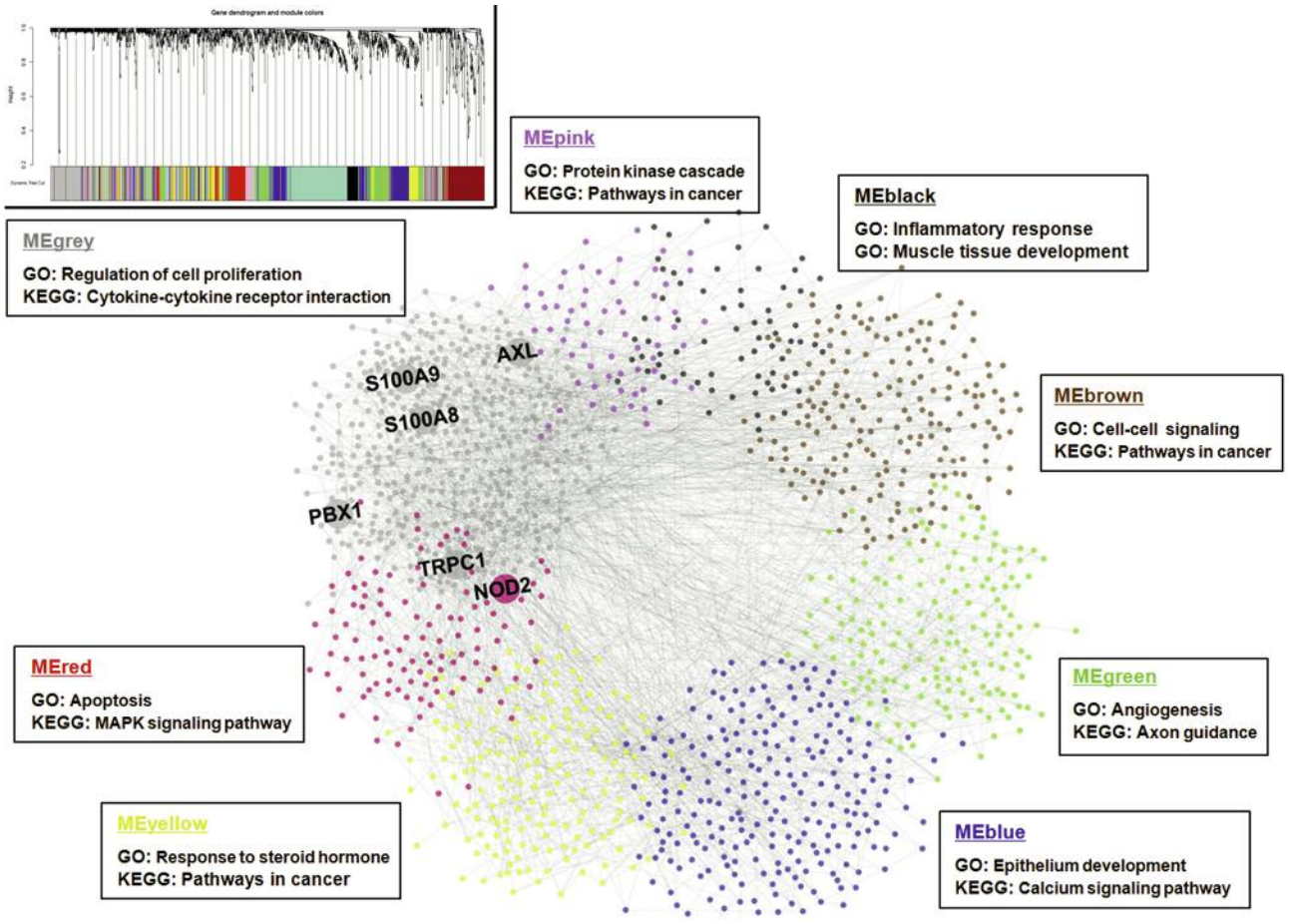

Figure 6. *Weighted gene correlation network analysis (WGCNA) of the total DEGs in the microarray dataset "GSE34228". In the GSE34228 dataset with the most samples, we constructed co-expression network of genes that have high correlation among the total 1033 DEGs, by using R package WGCNA. From the network, module detection analysis was performed by dynamic Tree Cut algorithm.*

Conflicts of Interest

None.

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References

1 Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C and Gottesman MM: Targeting multidrug resistance in cancer. Nat Rev Drug Discov *5*: 219-234, 2006.
2 Chen KG and Sikic BI: Molecular pathways: regulation and therapeutic implications of multidrug resistance. Clin Cancer Res *18*: 1863-1869, 2012.
3 Longley DB and Johnston PG: Molecular mechanisms of drug resistance. J Pathol *205*: 275-292, 2005.
4 Cappuzzo F, Finocchiaro G, Metro G, Bartolini S, Magrini E, Cancellieri A, Trisolini R, Castaldini L, Tallini G and Crino L: Clinical experience with gefitinib: an update. Crit Rev Oncol Hematol *58*: 31-45, 2006.
5 Roskoski R, Jr.: The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun *319*: 1-11, 2004.
6 Ono M and Kuwano M: Molecular mechanisms of epidermal growth factor receptor (EGFR) activation and response to gefitinib and other EGFR-targeting drugs. Clin Cancer Res *12*: 7242-7251, 2006.
7 Nurwidya F, Takahashi F, Murakami A, Kobayashi l, Kato M, Shukuya T, Tajima K, Shimada N and Takahashi K: Acquired resistance of non-small cell lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. Respir Investig *52*: 82-91, 2014.
8 Engelman JA and Janne PA: Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res *14*: 2895-2899, 2008.
9 Watanuki Z, Kosai H, Osanai N, Ogama N, Nochizuki M, amai K, Yamaguchi K, Satoh K, Fukuhara T, Maemondo M, Ichinose M, Nukiwa T and Tanaka N: Synergistic cytotoxicity of afatinib and cetuximab against *EGFR* T790M involves RAB11-dependent EGFR recycling. Biochem Biophys Res Commun *455*: 269-276, 2014.

10 Gottschling S, Schnabel PA, Herth FJ and Herpel E: Are we missing the target? Cancer stem cells and drug resistance in non-small cell lung cancer. Cancer Genomics Proteomics *9*: 275-286, 2012.
11 Ramasamy A, Mondry A, Holmes CC and Altman DG: Key issues in conducting a meta-analysis of gene expression microarray datasets. PLoS Med *5*: e184, 2008.
12 Li J, Li D, Wei X and Su Y: *In silico* comparative genomic analysis of two non-small cell lung cancer subtypes and their potentials for cancer classification. Cancer Genomics Proteomics *11*: 303-310, 2014.
13 Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, Rinehart C, Seidel B, Yee D, Arteaga CL and Engelman JA: Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest *118*: 2609-2619, 2008.
14 Terai H, Soejima K, Yasuda H, Nakayama S, Hamamoto J, Arai D, Ishioka K, Ohgino K, Ikemura S, Sato T, Yoda S, Satomi R, Naoki K and Betsuyaku T: Activation of the FGF2–FGFR1 autocrine pathway: a novel mechanism of acquired resistance to gefitinib in NSCLC. Mol Cancer Res *11*: 759-767, 2013.
15 Moher D, Liberati A, Tetzlaff J, Altman DG and the PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS MED *6*: e1000097, 2009.
16 Xia J, Fjell CD, Mayer ML, Pena OM, Wishart DS and Hancock RE: INMEX--a web-based tool for integrative meta-analysis of expression data. Nucleic Acids Res *41*: W63-70, 2013.
17 Song GG, Kim JH, Seo YH, Choi SJ, Ji JD and Lee YH: Meta-analysis of differentially expressed genes in primary Sjogren's syndrome by using microarray. Hum Immunol *75*: 98-104, 2014.
18 Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montoio J, Shao Q, Wright G and Morris Q: The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res *38*: W214-220, 2010.
19 Molina-Navarro MM, Trivino JC, Martinez-Dolz L, Lago F, Gonzalez-juanatey JR, Portoles M and Rivera M: Functional networks of nucleocytoplasmic transport-related genes differentiate ischemic and dilated cardiomyopathies. A new therapeutic opportunity. PLoS One *9*: e104709, 2014.
20 Gupta A, Schulze TG, Nagarajan V, Akula N, Corona W, Jiang XY, Hunter N, McMahon FJ and Detera-Wadleigh SD: Interaction networks of lithium and valproate molecular targets reveal a striking enrichment of apoptosis functional clusters and neurotrophin signaling. Pharmacogenomics J *12*: 328-341, 2012.
21 Nepusz T, Yu H and Paccanaro A: Detecting overlapping protein complexes in protein-protein interaction networks. Nat Methods *9*: 471-472, 2012.
22 Delgado AP, Hamid S, Brandao P and Narayanan R: A novel transmembrane glycoprotein cancer biomarker present in the X chromosome. Cancer Genomics Proteomics *11*: 81-92, 2014.
23 Zhang B and Horvath S: A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol *4*: Article17, 2005.
24 Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Dalla Favera R and Califano A: ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. BMC Bioinformatics *7(Suppl 1)*: S7, 2006.
25 Monteith GR, Davis FM and Roberts-Thomson SJ: Calcium channels and pumps in cancer: changes and consequences. J Biol Chem *287*: 31666-73, 2012.
26 Chen YF, Chen YT, Chiu WT and Shen MR: Remodeling of calcium signaling in tumor progression. J Biomed Sci *20*: 23, 2013.
27 Bryant JA, Finn RS, Slamon DJ, Cloughesy TF and Charles AC: EGF activates intracellular and intercellular calcium signaling by distinct pathways in tumor cells. Cancer Biol Ther *3*: 1243-1249, 2004.
28 Prevarskaya N, Zhang L and Barritt G: TRP channels in cancer. Biochim Biophys Acta *1772*: 937-946, 2007.
29 Tajeddine N and Gailly P: TRPC1 protein channel is major regulator of epidermal growth factor receptor signaling. J Biol Chem *287*: 16146-16157, 2012.
30 Srikrishna G: S100A8 and S100A9: new insights into their roles in malignancy. J Innate Immun *4*: 31-40, 2012.
31 Silva EJ, Argyris PP, Zou X, Ross KF and Herzberg MC: S100A8/A9 regulates MMP-2 expression and invasion and migration by carcinoma cells. Int J Biochem Cell Biol *55*: 279-287, 2014.
32 Duan L, Wu R, Ye L, Wang H, Yang X, Zhang Y, Chen X, Zuo G, Zhang Y, Weng Y, Luo J, Tang M, Shi Q, He T and Zhou L: S100A8 and S100A9 are associated with colorectal carcinoma progression and contribute to colorectal carcinoma cell survival and migration *via* Wnt/beta-catenin pathway. PLoS One *8*: e62092, 2013.
33 Werts C, Rubino S, Ling A, Girardin SE and Philpott DJ: Nod-like receptors in intestinal homeostasis, inflammation, and cancer. J Leukoc Biol *90*: 471-482, 2011.
34 Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T, Abdel-Rahman M, Wang X, Levine AD, Rho JK, Choi YJ, Choi CM, Kim SW, Jang SJ Park YS, Kim WS, Lee DH, Lee JS, Miller VA, Arcila M, Ladanyi M, Moonsamy P, Sawyers C, Boggon TJ, Ma PC, Costa C, Taron M, Rosell R, Halmos B and Bivona TG: Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 44: 852-860, 2012.
35 Thiaville MM, Stoeck A, Chen L, Wu RC, Magnani L, Oidtman J, Shih leM, Lupien M, Wang TL: Identification of PBX1 target genes in cancer cells by global mapping of PBX1-binding sites. PLoS One *7*: e36054, 2012.

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