PSMA7 and DUSP4 are promising druggable targets for treating Ovarian Neoplasms that control activity of PDX1, NR3C1 and ELK1 transcription factors on of differentially expressed genes

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Genome Enhancer release 2.3 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2021.1)

Abstract

In the present study we applied the software package "Genome Enhancer" to a multiomics data set that contains *transcriptomics and epigenomics* data. The study is done in the context of *Ovarian Neoplasms*. The goal of this pipeline is to identify potential drug targets in the molecular network that governs the studied pathological process. In the first step of analysis pipeline discovers transcription factors (TFs) that regulate genes activities in the pathological state. The activities of these TFs are controlled by so-called master regulators, which are identified in the second step of analysis. After a subsequent druggability checkup, the most promising master regulators are chosen as potential drug targets for the analyzed pathology. At the end the pipeline comes up with (a) a list of known drugs and (b) investigational active chemical compounds with the potential to interact with selected drug targets.

From the data set analyzed in this study, we found the following TFs to be potentially involved in the regulation of the differentially expressed genes: PDX1, NR3C1, SP1, ELK1, HSF1 and CEBPB. The subsequent network analysis suggested

- p/CAF
- DNA-PKcs
- MKP-2
- 26S proteasome

as the most promising molecular targets for further research, drug development and drug repurposing initiatives on the basis of identified molecular mechanism of the studied pathology. Having checked the actual druggability potential of the full list of identified targets, both, via information available in medical literature and via cheminformatics analysis of drug compounds, we have identified the following drugs as the most promising treatment candidates for the studied pathology: Pazopanib, Minocycline and 2,5,7-Trihydroxynaphthoquinone.

1. Introduction

Recording "-omics" data to measure gene activities, protein expression or metabolic events is becoming a standard approach to characterize the pathological state of an affected organism or tissue. Increasingly, several of these methods are applied in a combined approach leading to large "multiomics" datasets. Still the challenge remains how to reveal the underlying molecular mechanisms that render a given pathological state different from the norm. The disease-causing mechanism can be described by a re-wiring of the cellular regulatory network, for instance as a result of a genetic or epigenetic alterations influencing the activity of relevant genes. Reconstruction of the disease-specific regulatory networks can help identify potential master regulators of the respective pathological process. Knowledge about these master regulators can point to ways how to block a pathological regulatory cascade. Suppression of certain molecular targets as components of these cascades may stop the pathological process and cure the disease.

Conventional approaches of statistical "-omics" data analysis provide only very limited information about the causes of the observed phenomena and therefore contribute little to the understanding of the pathological molecular mechanism. In contrast, the "upstream analysis" method [1-4] applied here has been deviced to provide a casual interpretation of the data obtained for a pathology state. This approach comprises two major steps: (1) analysing promoters and enhancers of differentially expressed genes for the transcription factors (TFs) involved in their regulation and, thus, important for the process under study; (2) re-constructing the signaling pathways that activate these TFs and identifying master regulators at the top of such pathways. For the first step, the database TRANSFAC® [6] is employed together with the TF binding site identification algorithms Match [7] and CMA [8]. The second step involves the signal transduction database TRANSPATH® [9] and special graph search algorithms [10] implemented in the software "Genome Enhancer".

The "upstream analysis" approach has now been extended by a third step that reveals known drugs suitable to inhibit (or activate) the identified molecular targets in the context of the disease under study. This step is performed by using information from HumanPSD™ database [5]. In addition, some known drugs and investigational active chemical compounds are subsequently predicted as potential ligands for the revealed molecular targets. They are predicted using a pre-computed database of spectra of biological activities of chemical compounds of a library of 2245 known drugs and investigational chemical compounds from HumanPSD™ database. The spectra of biological activities for these compounds are computed using the program PASS on the basis of a (Q)SAR approach [11-13]. These predictions can be used for the research purposes - for further drug development and drug repurposing initiatives.

2. Data

For this study the following experimental data was used:

Table 1. Experimental datasets used in the study

File name	Data type
GSM385721.CEL	Transcriptomics
GSM385722.CEL	Transcriptomics
GSM385723.CEL	Transcriptomics
GSM385724.CEL	Transcriptomics
GSM385725.CEL	Transcriptomics
GSM385726.CEL	Transcriptomics
GSM385727.CEL	Transcriptomics
GSM385728.CEL	Transcriptomics
GSM385729.CEL	Transcriptomics
GSM385730.CEL	Transcriptomics
GSM385747_CpG_NM.fixed.hg38.top300	Epigenomics

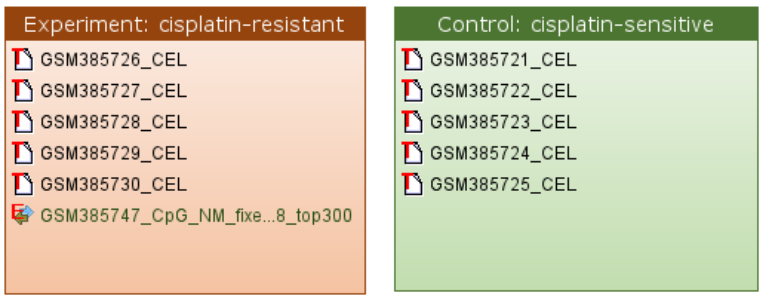


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

3. Results

We have compared the following conditions: Experiment: cisplatin-resistant *versus* Control: cisplatin-sensitive.

3.1. Identification of target genes

In the first step of the analysis ***target genes*** were identified from the uploaded experimental data. We applied the Limma tool (R/Bioconductor package integrated into our pipeline) and compared gene expression in the following sets: "Experiment: cisplatin-resistant" with "Control: cisplatin-sensitive". Limma calculated the LogFC (the logarithm to the base 2 of the fold change between different conditions), the p-value and the adjusted p-value (corrected for multiple testing) of the observed fold change. As a result, we detected 13720 upregulated genes (LogFC>0) out of which 9237 genes were found as significantly upregulated (p-value<0.1) and 13600 downregulated genes (LogFC<0) out of which 9071 genes were significantly downregulated (p-value<0.1). See tables below for the top significantly up- and downregulated genes. Below we call **target genes** the full list of up- and downregulated genes revealed in our analysis (see tables in Supplementary section).

*Table 2. Top ten significant **up-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.*
See full table →

ID	Gene symbol	Gene description	logFC	P.Value	adj.P.Val
ENSG00000123700	KCNJ2	potassium inwardly rectifying channel subfamily J member 2	5.31	2.14E-15	5.32E-12
ENSG00000064218	DMRT3	doublesex and mab-3 related transcription factor 3	5.17	3.71E-16	1.48E-12
ENSG00000099139	PCSK5	proprotein convertase subtilisin/kexin type 5	4.46	1.36E-12	5.01E-10
ENSG00000197705	KLHL14	kelch like family member 14	3.68	6.09E-15	9.4E-12
ENSG00000103449	SALL1	spalt like transcription factor 1	3.4	6.17E-12	1.43E-9
ENSG00000138378	STAT4	signal transducer and activator of transcription 4	3.39	1.15E-11	2.4E-9
ENSG00000164692	COL1A2	collagen type I alpha 2 chain	3.3	9.02E-15	1.03E-11
ENSG00000133083	DCLK1	doublecortin like kinase 1	3.29	8.04E-15	1.03E-11
ENSG00000126950	TMEM35A	transmembrane protein 35A	3.16	4.71E-15	8.05E-12
ENSG00000116132	PRRX1	paired related homeobox 1	3.15	3.8E-14	3.25E-11

*Table 4. Top ten significant **down-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.*
See full table →

ID	Gene symbol	Gene description	logFC	P.Value	adj.P.Val
ENSG00000149968	MMP3	matrix metallopeptidase 3	-6.61	2.63E-18	5.42E-14
ENSG00000127324	TSPAN8	tetraspanin 8	-6.08	2.63E-14	2.57E-11
ENSG00000139292	LGR5	leucine rich repeat containing G protein-coupled receptor 5	-5.52	2.04E-16	1.4E-12
ENSG00000153233	PTPRR	protein tyrosine phosphatase receptor type R	-5.28	3.52E-16	1.48E-12
ENSG00000169908	TM4SF1	transmembrane 4 L six family member 1	-4.65	3.97E-18	5.42E-14
ENSG00000106511	MEOX2	mesenchyme homeobox 2	-4.63	1.53E-15	4.66E-12
ENSG00000060718	COL11A1	collagen type XI alpha 1 chain	-4.53	3.92E-14	3.25E-11
ENSG00000163359	COL6A3	collagen type VI alpha 3 chain	-4.52	2.87E-17	2.61E-13
ENSG00000166670	MMP10	matrix metallopeptidase 10	-4.28	2.96E-15	6.32E-12
ENSG00000145431	PDGFC	platelet derived growth factor C	-4.09	5.02E-16	1.71E-12

3.2. Regulatory regions of target genes

We mapped the uploaded Epigenomic peaks on the ***target genes*** and selected those peaks only that were found located in the body of the gene (in exons or introns of the genes) or in the 5000 nucleotide long flanking regions of the genes. In the tables below we demonstrate localization of such potential regulatory regions in the top up-regulated and down-regulated genes.

*Table 3. Top 3 **up-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive with epigenomic peaks.*
See full table →

ID	Gene symbol	Gene schematic representation
ENSG00000260774	AC021087.3	
ENSG00000027075	PRKCH	
ENSG00000186684	CYP27C1	

*Table 5. Top 7 **down-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive with epigenomic peaks.*
See full table →

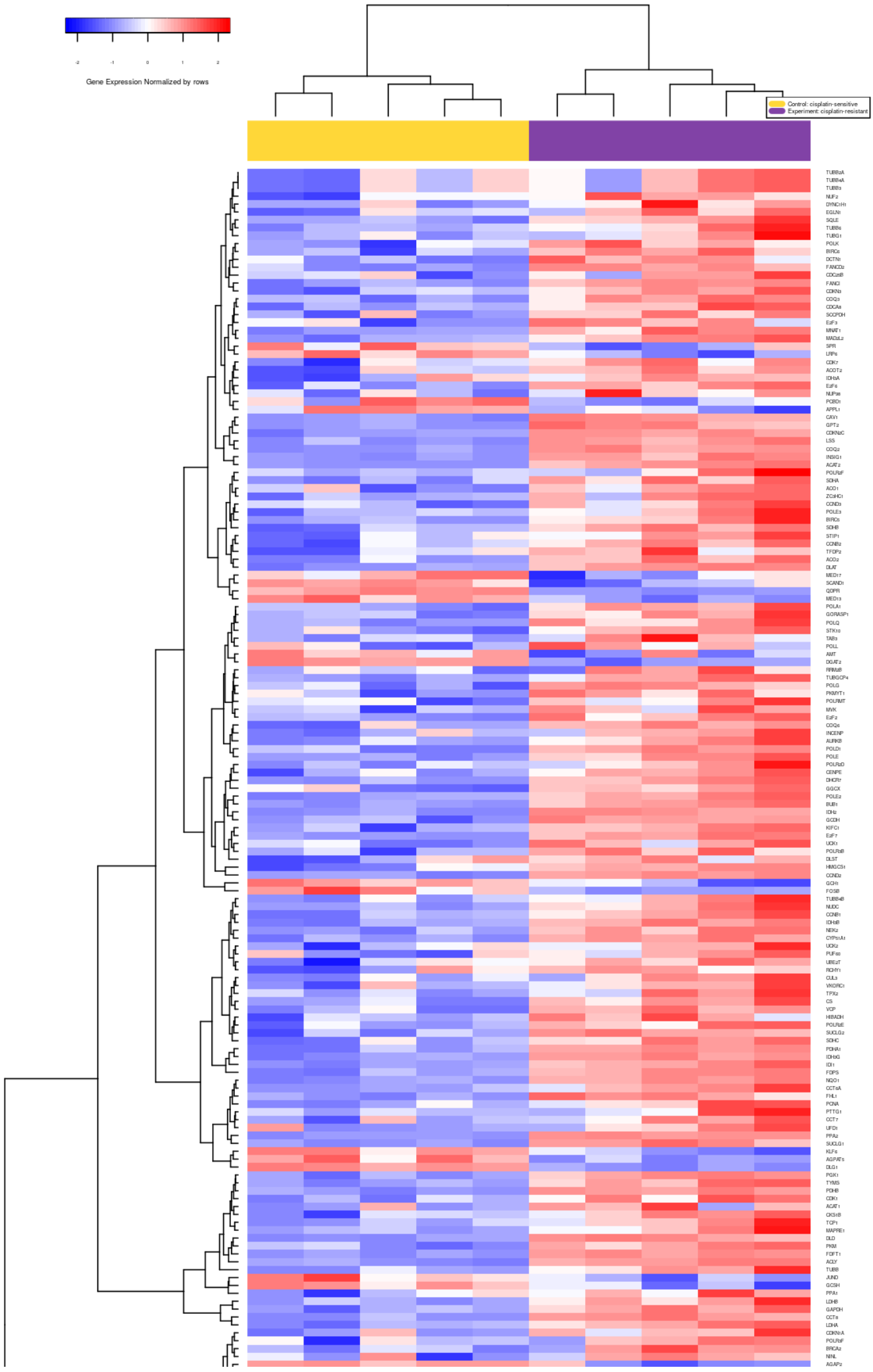
ID	Gene symbol	Gene schematic representation
ENSG00000170558	CDH2	
ENSG00000197921	HES5	
ENSG00000197822	OCLN	
ENSG00000146648	EGFR	
ENSG00000145476	CYP4V2	
ENSG00000237765	FAM200B	
ENSG00000118495	PLAGL1	

3.3. Functional classification of genes

A functional analysis of differentially expressed genes was done by mapping the significant up-regulated and significant down-regulated genes to several known ontologies, such as Gene Ontology (GO), disease ontology (based on HumanPSD™ database) and the ontology of signal transduction and metabolic pathways from the TRANSPATH® database. Statistical significance was computed using a binomial test.
Figures 3-8 show the most significant categories.

Heatmap of differentially expressed genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive

A heatmap of all differentially expressed genes playing a potential regulatory role in the system (enriched in TRANSPATH® pathways) is presented in Figure 2.


-2
-1
0
1
2
Gene Expression Normalized by rows
Control: cisplatin-sensitive
Experiment: cisplatin-resistant
TUBB3
NUF2
DYNC1H1
EGLN1
SQLE
TUBB6
TUBG1
POLK
BIRC6
DCTN1
FANCD2
CDC20B
FANCI
CDKN3
COQ3
CDCA8
SCCPDH
EIF3
MNAT1
MADLL2
SPR
URB6
CDK7
ACOT2
IDH3A
EIF6
NUP98
PGBD1
APPL1
CAV1
GPT2
CDKN2C
LSS
COQ2
INSIG1
ACAT2
POLR2F
SDHA
ACO1
ZC3HC1
CCND3
POLE3
BIRC5
SDHB
STIP1
CCNB2
TFDP2
ACO2
DLAT
MED17
SCAND1
QDPR
MED13
POLA1
GORASP1
POLQ
STK10
TAB3
POLL
AMT
DGAT2
RRM2B
TUBGCP4
POLG
PKMYT1
POLR1MT
MVK
EIF2
COQ6
INCENP
AURKB
POLD1
POLE
POLR2D
CENPE
DHCR7
GGCX
POLE2
BUB1
IDH2
GCDH
KIFC1
EIF7
UCK1
POLR2B
DLST
HMGCS1
CCND2
GCH1
FOSB
TUBB4B
NUDC
CCNB1
IDH3B
NEK2
CYP51A1
UCK2
PUF60
UBE2T
RCHY1
CUL3
VRK3
TPX2
CS
VCP
HIBADH
POLR2E
SUCLG2
SDHC
PDHA1
IDH3G
IDI1
FDPS
NQO1
CCT6A
FHL1
PCNA
PTTG1
CCT7
UFD1
PPA2
SUCLG1
KLF6
AGPAT5
DLG1
PGK1
TYMS
PDHB
CDK1
ACAT1
CKSH2
TCP1
MAPRE1
DLD
PKM
FDFT1
ACLY
TUBB
JUND
GCSH
PPA1
LDHB
GAPDH
CCT8
LDHA
CDKN1A
POLR2F
BRCA2
NINL
AGAP2

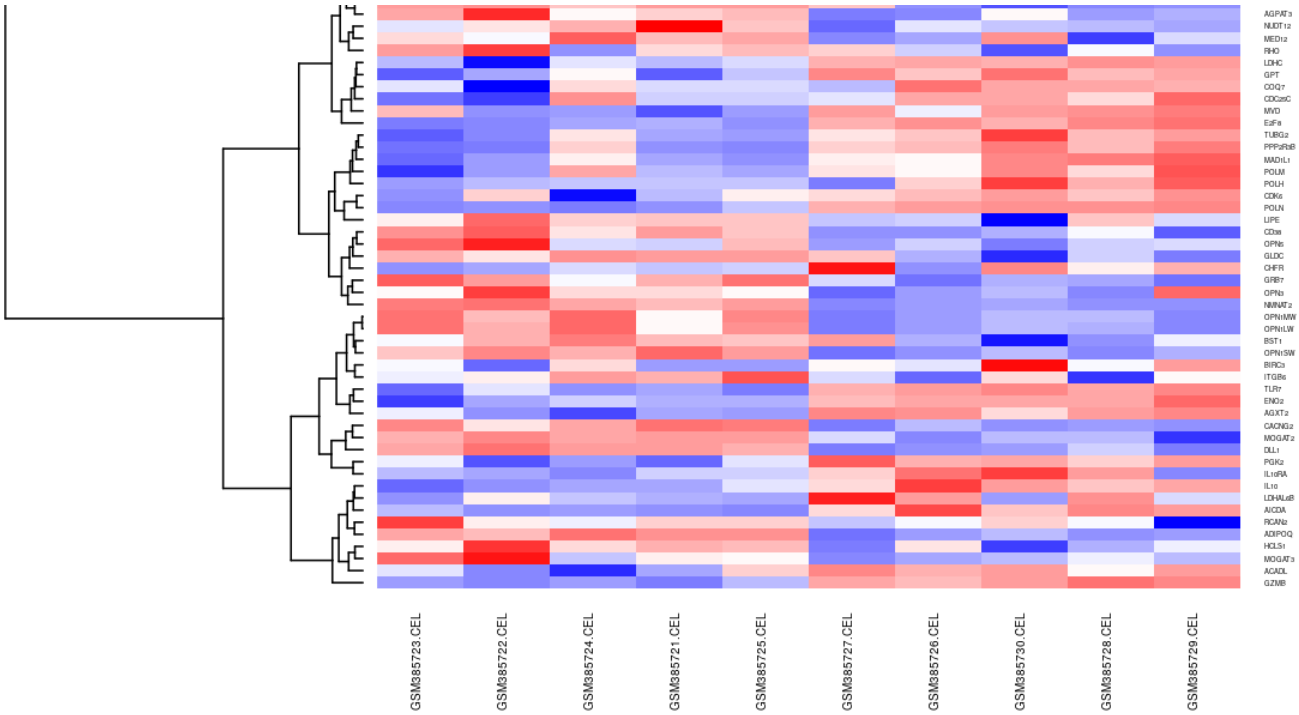


Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.
See full diagram →

Up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive:

9237 significant up-regulated genes were taken for the mapping.

GO (biological process)

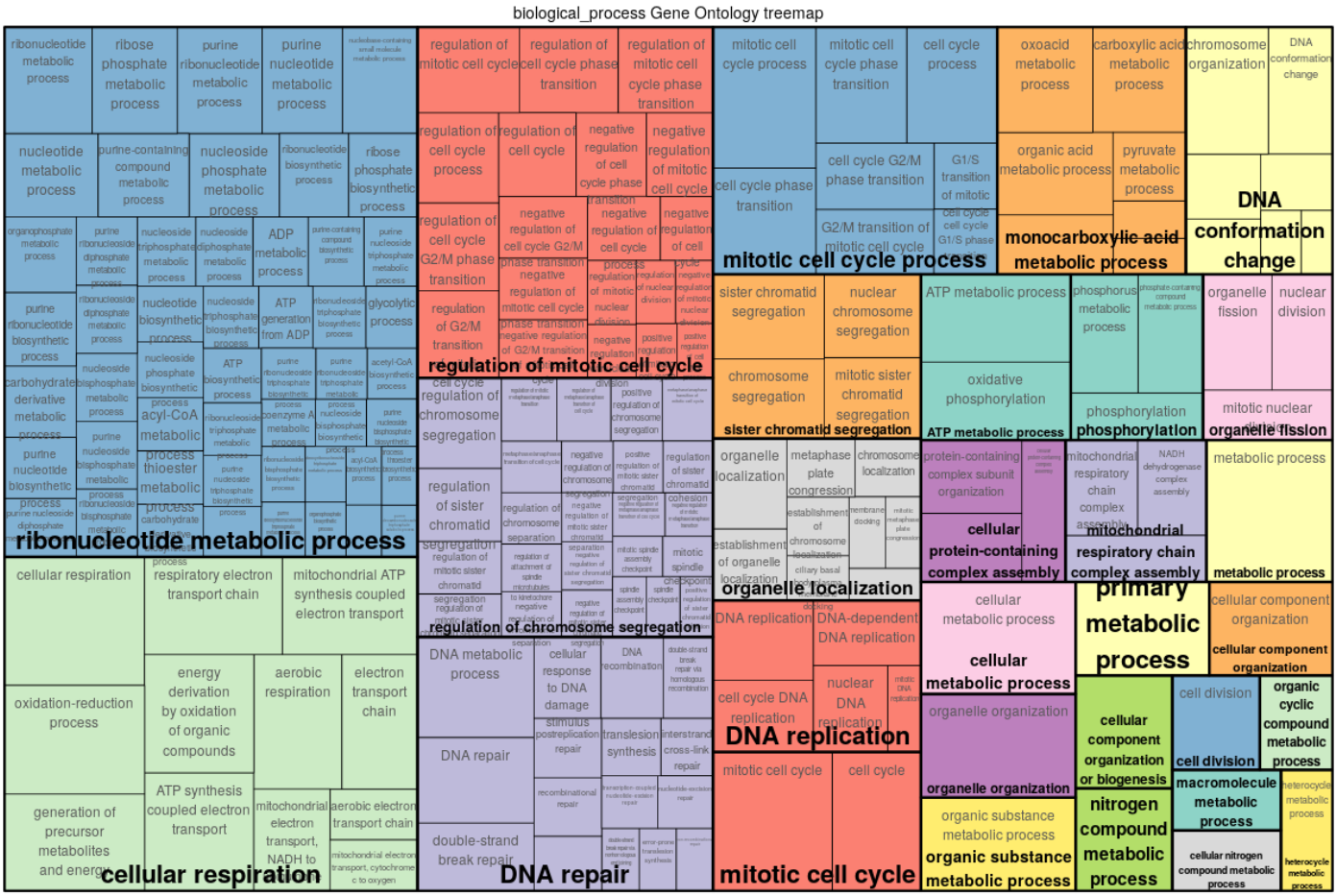


Figure 3. Enriched GO (biological process) of up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.
Full classification →

TRANSPATH® Pathways (2021.1)

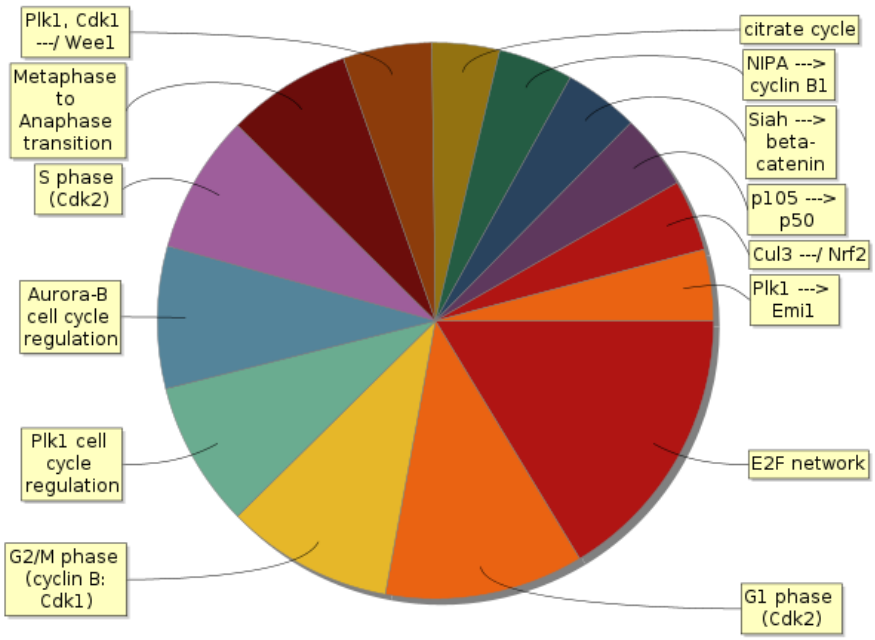


Figure 4. Enriched TRANSPATH® Pathways (2021.1) of up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.
Full classification →

HumanPSD(TM) disease (2021.1)

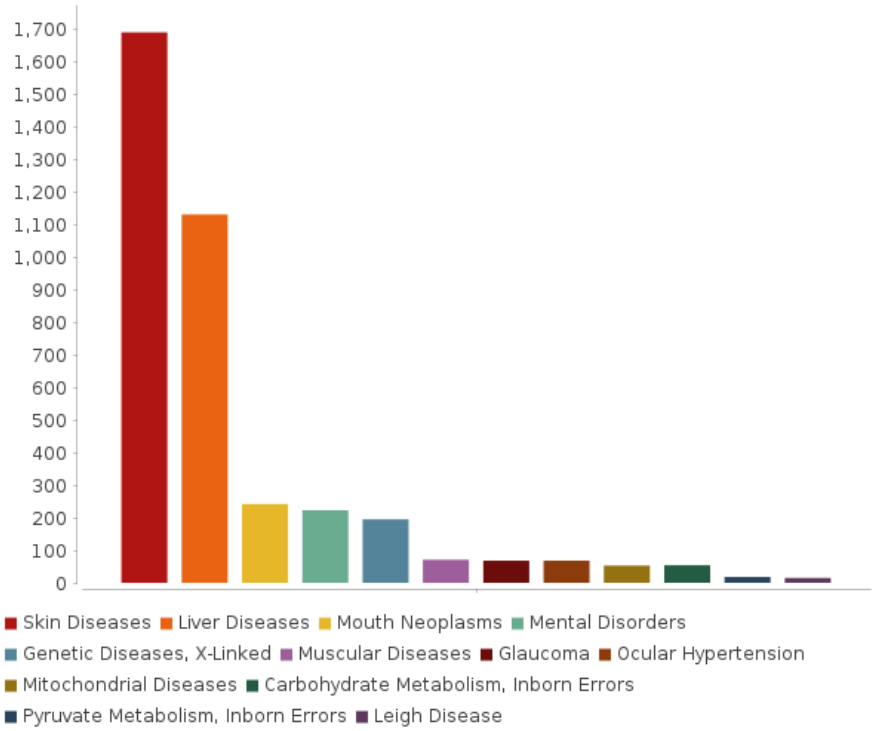


Figure 5. Enriched HumanPSD(TM) disease (2021.1) of up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.
Full classification →

Down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive:

9071 significant down-regulated genes were taken for the mapping.

GO (biological process)

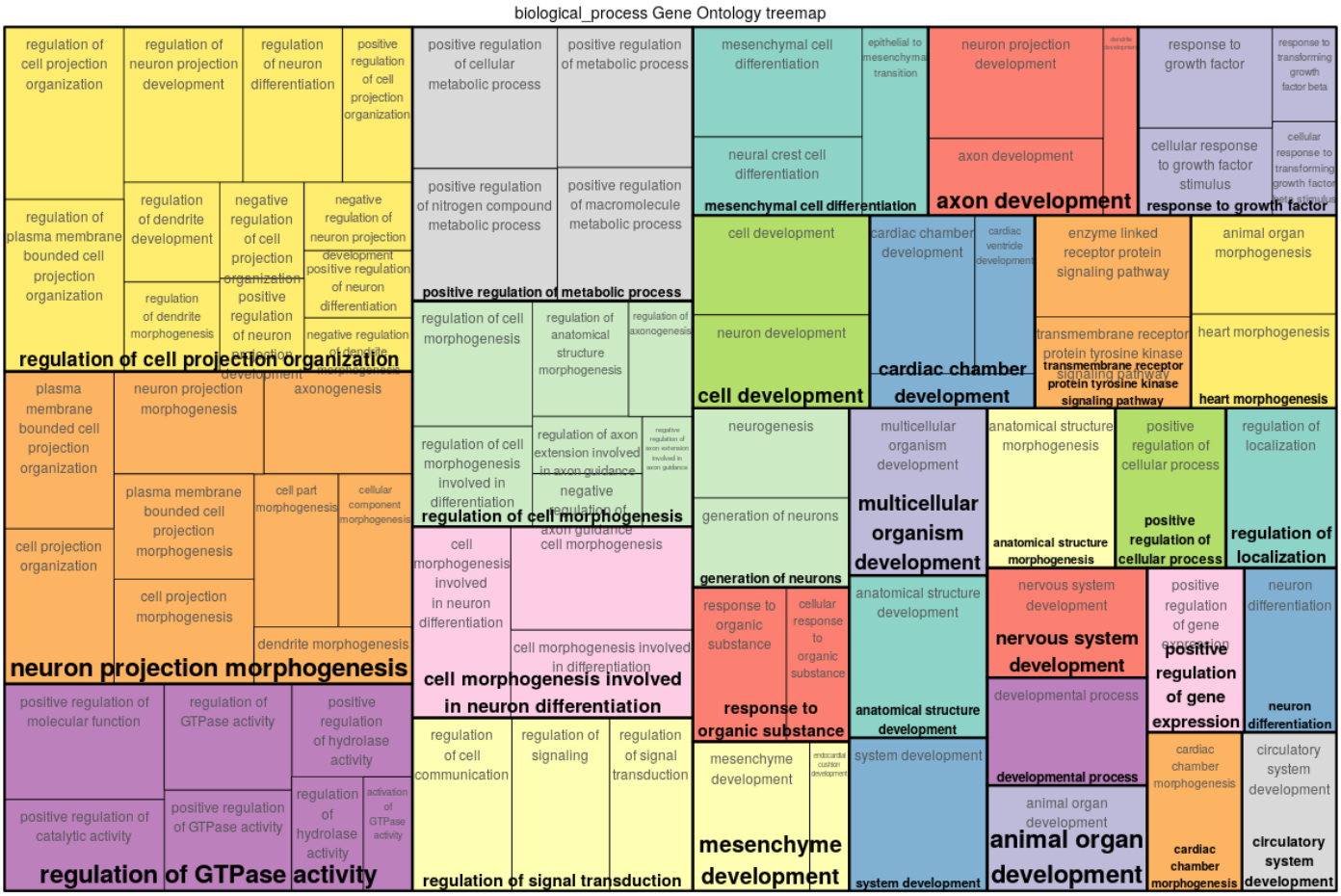


Figure 6. Enriched GO (biological process) of down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.
Full classification →

TRANSPATH® Pathways (2021.1)

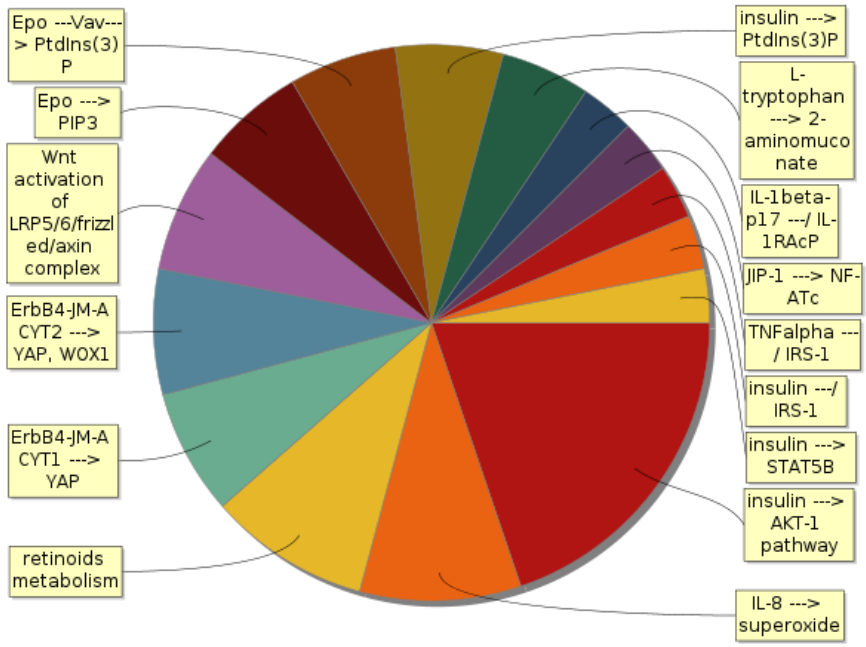


Figure 7. Enriched TRANSPATH® Pathways (2021.1) of down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive.
Full classification →

HumanPSD(TM) disease (2021.1)

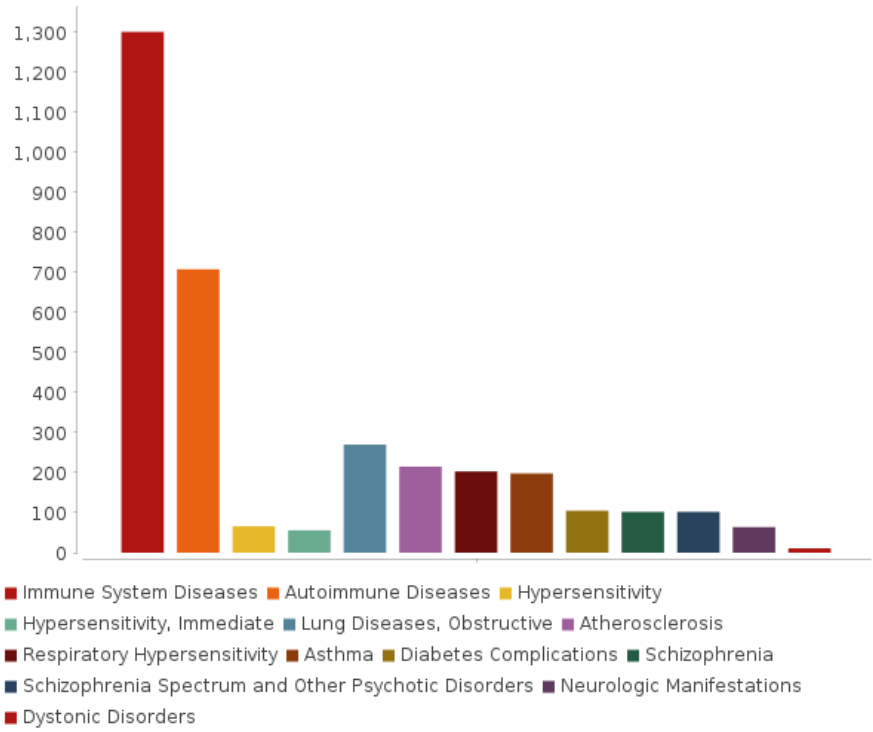
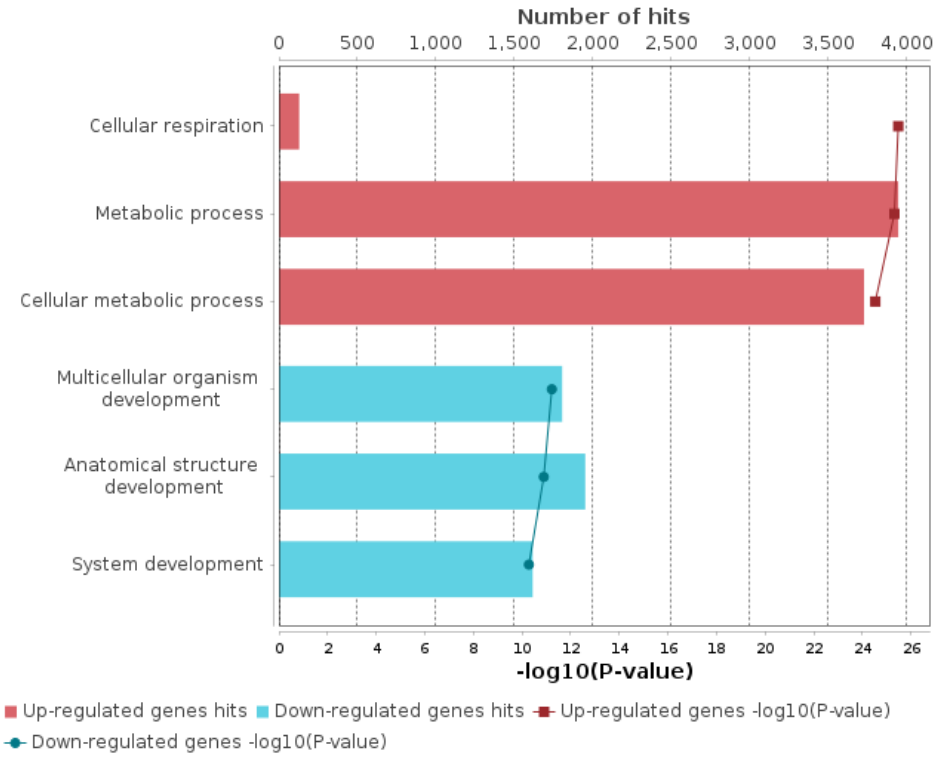


Figure 8. Enriched HumanPSD(TM) disease (2021.1) of down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.
Full classification →

The result of overall Gene Ontology (GO) analysis of the differentially expressed genes of the studied pathology can be summarized by the following diagram, revealing the most significant functional categories overrepresented among the observed (differentially expressed genes):



3.4. Analysis of enriched transcription factor binding sites and composite modules

In the next step a search for transcription factors binding sites (TFBS) was performed in the regulatory regions of the ***target genes*** by using the TF binding motif library of the TRANSFAC® database. We searched for so called **composite modules** that act as potential condition-specific **enhancers** of the ***target genes*** in their upstream regulatory regions (-1000 bp upstream of transcription start site (TSS)) and identify transcription factors regulating activity of the genes through such **enhancers**.

Classically, **enhancers** are defined as regions in the genome that increase transcription of one or several genes when inserted in either orientation at various distances upstream or downstream of the gene [8]. Enhancers typically have a length of several hundreds of nucleotides and are bound by multiple transcription factors in a cooperative manner [9].

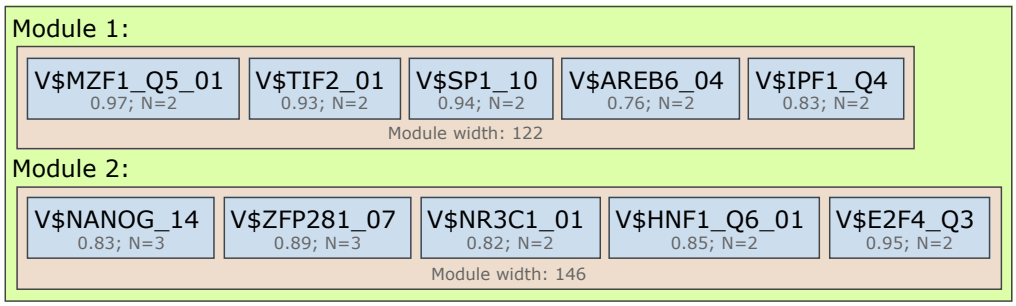
In the current work we use the Epigenomics data from the track(s) "GSM385747_CpG_NM.fixed.hg38.top300" to predict positions of potential ***enhancers*** regulating the differentially expressed genes revealed by comparative transcriptomics analysis. We took genomic regions -550bp upstream and 550bp downstream from the middle point of each interval of the track and check if these regions are located inside the 5kb flanking arias of the differentially expressed genes (or inside the body of the genes). In such cases, these genomic regions are used for the search for potential condition-specific enhancers. In all other cases when the differentially expressed genes did not contain epigenomic peaks in their body or in the 5kb flanking regions we used the upstream regulatory regions of these genes (-1000bp upstream and 100bp downstream of TSS) for the search for condition-specific enhancers.

We applied the Composite Module Analyst (CMA) [8] method to detect such potential enhancers, as targets of multiple TFs bound in a cooperative manner to the regulatory regions of the genes of interest. CMA applies a genetic algorithm to construct a generalized model of the enhancers by specifying combinations of TF motifs (from TRANSFAC®) whose sites are most frequently clustered together in the regulatory regions of the studied genes. CMA identifies the transcription factors that through their cooperation provide a synergistic effect and thus have a great influence on the gene regulation process.

Enhancer model potentially involved in regulation of target genes (up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive).

To build the most specific composite modules we choose top 300 significant up-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all up-regulated genes.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.



Model score (-p*log10(pval)): 14.84
Wilcoxon p-value (pval): 2.50e-30
Penalty (p): 0.501
Average yes-set score: 7.29
Average no-set score: 5.77
AUC: 0.74
Middle-point: 6.70
False-positive: 36.40%
False-negative: 26.00%

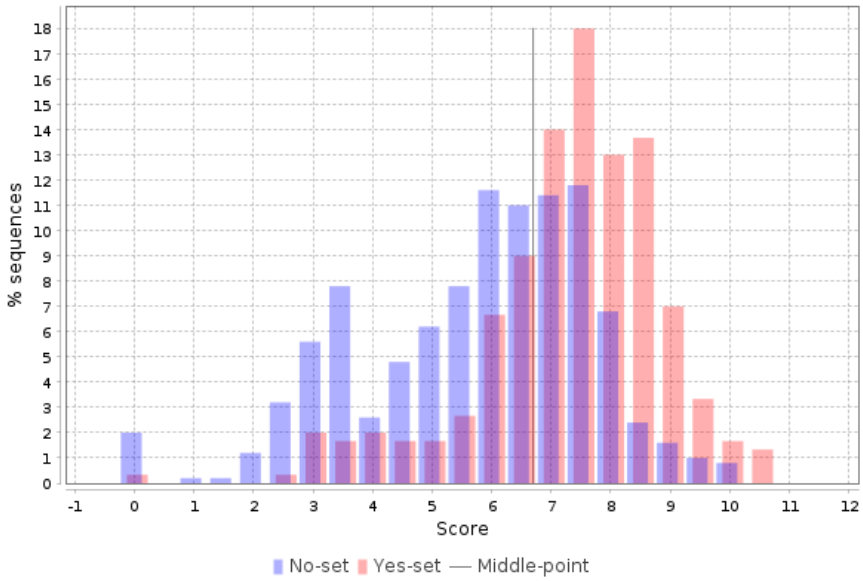


Table 6. List of top ten up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*
See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000100364	KIAA0930	KIAA0930	11.96	MZF-1(h), Sp1(h), ZBP99(h), ZEB1(h), nanog(h), E2F-4(h)
ENSG00000232148	FMO11P	flavin containing dimethylaniline monoxygenase 11, pseudogene	11.88	MZF-1(h), ZBP99(h), ZEB1(h), ipf1(h), TIF2(h), HNF-1alpha(h),HNF-1beta(h), nanog(h)...
ENSG00000110723	EXPH5	exophilin 5	11.87	ipf1(h), nanog(h), ZEB1(h), ZBP99(h), MZF-1(h), Sp1(h), E2F-4(h)...
ENSG00000128536	CDHR3	cadherin related family member 3	11.69	ipf1(h), TIF2(h), ZEB1(h), MZF-1(h), nanog(h), ZBP99(h)
ENSG00000203710	CR1	complement C3b/C4b receptor 1 (Knops blood group)	11.66	HNF-1alpha(h),HNF-1beta(h), TIF2(h), ZEB1(h), nanog(h), ipf1(h), ZBP99(h), MZF-1(h)
ENSG00000197576	HOXA4	homeobox A4	11.32	nanog(h), ipf1(h), ZEB1(h), Sp1(h), ZBP99(h), MZF-1(h)
ENSG00000113971	NPHP3	nephrocystin 3	11.3	ZEB1(h), nanog(h), MZF-1(h), ZBP99(h), Sp1(h), E2F-4(h), ipf1(h)
ENSG00000137845	ADAM10	ADAM metallopeptidase domain 10	11.28	ZBP99(h), nanog(h), MZF-1(h), E2F-4(h), ZEB1(h), TIF2(h), GR(h)...
ENSG00000111796	KLRB1	killer cell lectin like receptor B1	11.23	GR(h), HNF-1alpha(h),HNF-1beta(h), TIF2(h), ipf1(h), ZEB1(h), nanog(h), ZBP99(h)...
ENSG00000154654	NCAM2	neural cell adhesion molecule 2	11.21	nanog(h), ZBP99(h), GR(h), E2F-4(h), ZEB1(h), MZF-1(h), Sp1(h)

Enhancer model potentially involved in regulation of target genes (down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive).

To build the most specific composite modules we choose top 300 significant down-regulated genes as the input of CMA algorithm. The obtained CMA model is then applied to compute CMA score for all down-regulated genes.

The model consists of 2 module(s). Below, for each module the following information is shown:
- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

Module 1:

V$HSF1_01	V$FOXO1ETV4_02	V$CEBP_Q3	V$SNF2H_01	V$TIF2_01
0.97; N=2	0.86; N=1	0.99; N=2	0.82; N=3	0.90; N=2

Module width: 136

Module 2:

V$WT1_Q6_02	V$POU2F1ELK1_01	V$HSF1_Q5_01	V$HNF1_Q6	V$E2F2_11	V$FOXO1ETV4_02
0.94; N=1	0.76; N=3	0.95; N=1	0.89; N=1	0.81; N=3	0.86; N=3

Module width: 150

Model score (-p*log10(pval)): 17.69
Wilcoxon p-value (pval): 4.85e-37
Penalty (p): 0.487
Average yes-set score: 4.27
Average no-set score: 2.63
AUC: 0.77
Middle-point: 3.72
False-positive: 23.60%
False-negative: 31.33%

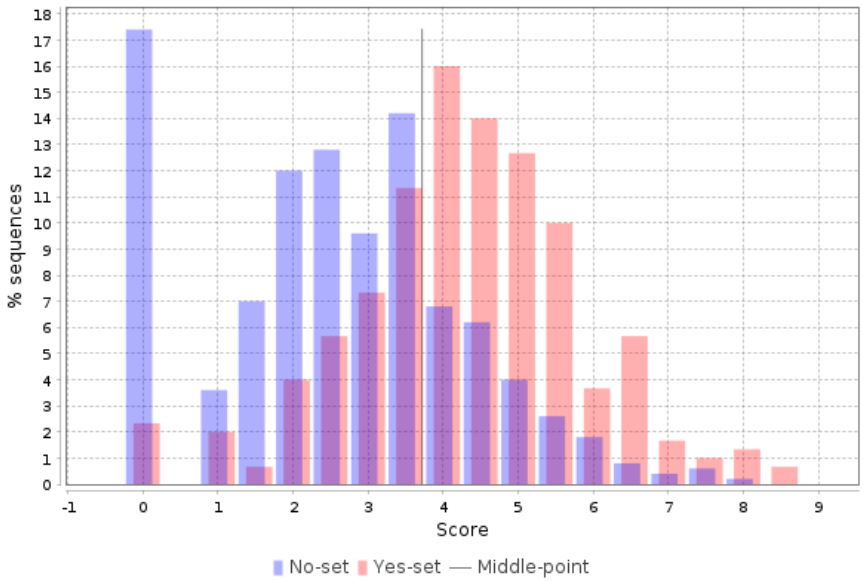


Table 7. List of top ten down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000151067	CACNA1C	calcium voltage-gated channel subunit alpha1 C	9.67	C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), TIF2(h), FOXO1A(h),PEA3(h), HSF1(h), Elk-1(h),POU2F1(h), HNF-1alpha(h),HNF-1beta(h)
ENSG00000120306	CYSTM1	cysteine rich transmembrane module containing 1	9.6	FOXO1A(h),PEA3(h), TIF2(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), HSF1(h), SNF2H(h), WT1(h), E2F-2(h)
ENSG00000088205	DDX18	DEAD-box helicase 18	9.14	SNF2H(h), HSF1(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), Elk-1(h),POU2F1(h), E2F-2(h), FOXO1A(h),PEA3(h)
ENSG00000204104	TRAF3IP1	TRAF3 interacting protein 1	9.12	HSF1(h), TIF2(h), FOXO1A(h),PEA3(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), Elk-1(h),POU2F1(h), E2F-2(h), WT1(h)
ENSG00000274425	AC114271.1	novel transcript, antisense to ICAM3	9.01	C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), TIF2(h), HSF1(h), FOXO1A(h),PEA3(h), SNF2H(h), E2F-2(h), WT1(h)...
ENSG00000271860	AL589740.1	novel transcript	8.79	TIF2(h), FOXO1A(h),PEA3(h), WT1(h), HSF1(h), E2F-2(h), Elk-1(h),POU2F1(h)
ENSG00000135334	AKIRIN2	akirin 2	8.61	Elk-1(h),POU2F1(h), WT1(h), E2F-2(h), TIF2(h), HSF1(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h)
ENSG00000246985	SOCS2-AS1	SOCS2 antisense RNA 1	8.53	WT1(h), Elk-1(h),POU2F1(h), E2F-2(h), HSF1(h), FOXO1A(h),PEA3(h), TIF2(h)
ENSG00000147180	ZNF711	zinc finger protein 711	8.51	HSF1(h), SNF2H(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), TIF2(h), FOXO1A(h),PEA3(h), E2F-2(h)
ENSG00000277842	MIR6811	microRNA 6811	8.46	SNF2H(h), C/EBPalpha(h),C/EBPbeta(h),C/EBPdelta(h),C/EBPepsilon(h),C/EBPgamma(h), TIF2(h), FOXO1A(h),PEA3(h), E2F-2(h), HSF1(h)

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 11 and 16 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 8-9).

Table 8. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive). ***Yes-No ratio*** *is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions.* ***Regulatory score*** *is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

See full table →

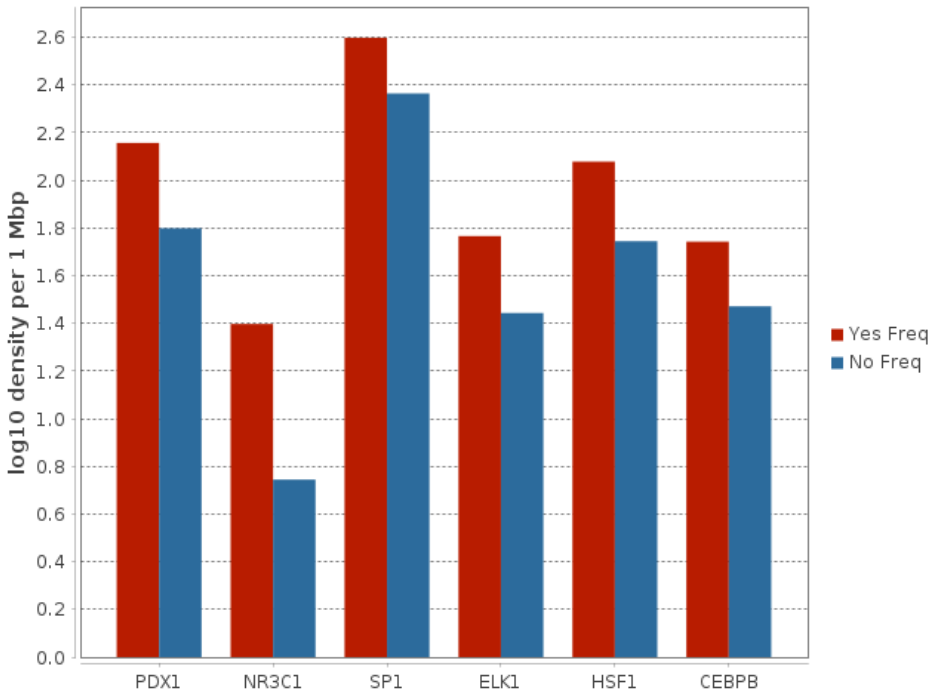
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO0000007664	PDX1	pancreatic and duodenal homeobox 1	4.12	2.28
MO0000031266	NR3C1	nuclear receptor subfamily 3 group C member 1	3.59	4.49
MO0000033308	SP1	Sp1 transcription factor	3.47	1.71
MO0000139677	ZEB1	zinc finger E-box binding homeobox 1	3.1	1.1
MO0000023603	E2F4	E2F transcription factor 4	2.89	2.4
MO0000028758	ZNF281	zinc finger protein 281	2.8	1.5
MO0000134485	NANOG	Nanog homeobox	2.77	2.76
MO0000026464	NCOA2	nuclear receptor coactivator 2	2.59	1.18
MO0000082618	HNF1A	HNF1 homeobox A	2.03	2.84
MO0000082711	HNF1B	HNF1 homeobox B	0	1.6

Table 9. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive). ***Yes-No ratio*** *is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions.* ***Regulatory score*** *is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).*

See full table →

ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO0000019544	ELK1	ETS transcription factor ELK1	2.3	2.1
MO0000033378	HSF1	heat shock transcription factor 1	2.18	2.16
MO0000019381	CEBPB	CCAAT enhancer binding protein beta	1.98	1.87
MO0000019418	CEBPA	CCAAT enhancer binding protein alpha	1.96	2.41
MO0000034454	FOXO1	forkhead box O1	1.74	3.04
MO0000046009	ETV4	ETS variant transcription factor 4	1.72	3.65
MO0000002641	CEBPD	CCAAT enhancer binding protein delta	1.71	1.4
MO0000025003	POU2F1	POU class 2 homeobox 1	1.71	9.96
MO0000004278	E2F2	E2F transcription factor 2	1.41	1.51
MO0000125339	SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	1.4	2.39

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: PDX1, NR3C1, SP1, ELK1, HSF1 and CEBPB.



3.5. Finding master regulators in networks

In the second step of the upstream analysis common regulators of the revealed TFs were identified. These master regulators appear to be the key candidates for therapeutic targets as they have a master effect on regulation of intracellular pathways that activate the pathological process of our study. The identified master regulators are shown in Tables 10-11.

*Table 10. Master regulators that may govern the regulation of **up-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and epigenomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO0000030927	DNA-PKcs(h)	PRKDC	protein kinase, DNA-activated, catalytic subunit	0.58	128
MO0000032652	MKP-2(h)	DUSP4	dual specificity phosphatase 4	1.17	140
MO0000020249	26S proteasome(h)	PSMA7, PSMC2, PSMC3, PSMC5, PSMD4, PSMD5	proteasome 20S subunit alpha 7, proteasome 26S subunit, ATPase 2, proteasome 26S subunit, ATPase 3, ...	0.44	189
MO0000056491	p/CAF(h)	KAT2B	lysine acetyltransferase 2B	0.63	192
MO0000019376	Cot(h)	MAP3K8	mitogen-activated protein kinase kinase kinase 8	1.87	251
MO0000151603	DNA-PKcs(h):Ku70(h):Ku80(h)	PRKDC, XRCC5, XRCC6	X-ray repair cross complementing 5, X-ray repair cross complementing 6, protein kinase, DNA-activate...	0.58	273
MO0000041170	EAC(h)	CYLD	CYLD lysine 63 deubiquitinase	1.06	284
MO0000080193	DNA-PKcs-isoform1(h)	PRKDC	protein kinase, DNA-activated, catalytic subunit	0.58	288
MO0000023409	p/CAF(h)	KAT2B	lysine acetyltransferase 2B	0.63	295
MO0000092591	Cdk1-isoform1(h):cyclinB1-isoform1(h)	CCNB1, CDK1	cyclin B1, cyclin dependent kinase 1	0.85	303

*Table 11. Master regulators that may govern the regulation of **down-regulated** genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and epigenomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO0000038172	plk2(h)	PLK2	polo like kinase 2	-2.44	48
MO0000154924	plk2(h)	PLK2	polo like kinase 2	-2.44	77
MO0000022222	MKP-1(h)	DUSP1	dual specificity phosphatase 1	-1.21	108
MO0000083769	MKP-1(h)	DUSP1	dual specificity phosphatase 1	-1.21	212
MO0000129772	PTP-SL(h)	PTPRR	protein tyrosine phosphatase receptor type R	-5.28	215
MO0000005412	Fyn(h)	FYN	FYN proto-oncogene, Src family tyrosine kinase	-0.54	229
MO0000007821	JNK1(h)	MAPK8	mitogen-activated protein kinase 8	-0.41	235
MO0000019070	XIAP(h)	XIAP	X-linked inhibitor of apoptosis	-0.58	276
MO0000035083	CnAalpha(h)	PPP3CA	protein phosphatase 3 catalytic subunit alpha	-0.59	294
MO0000042839	ptpn21(h)	PTPN21	protein tyrosine phosphatase non-receptor type 21	-1.32	302

The intracellular regulatory pathways controlled by the above-mentioned master regulators are depicted in Figures 9 and 10. These diagrams display the connections between identified transcription factors, which play important roles in the regulation of differentially expressed genes, and selected master regulators, which are responsible for the regulation of these TFs.

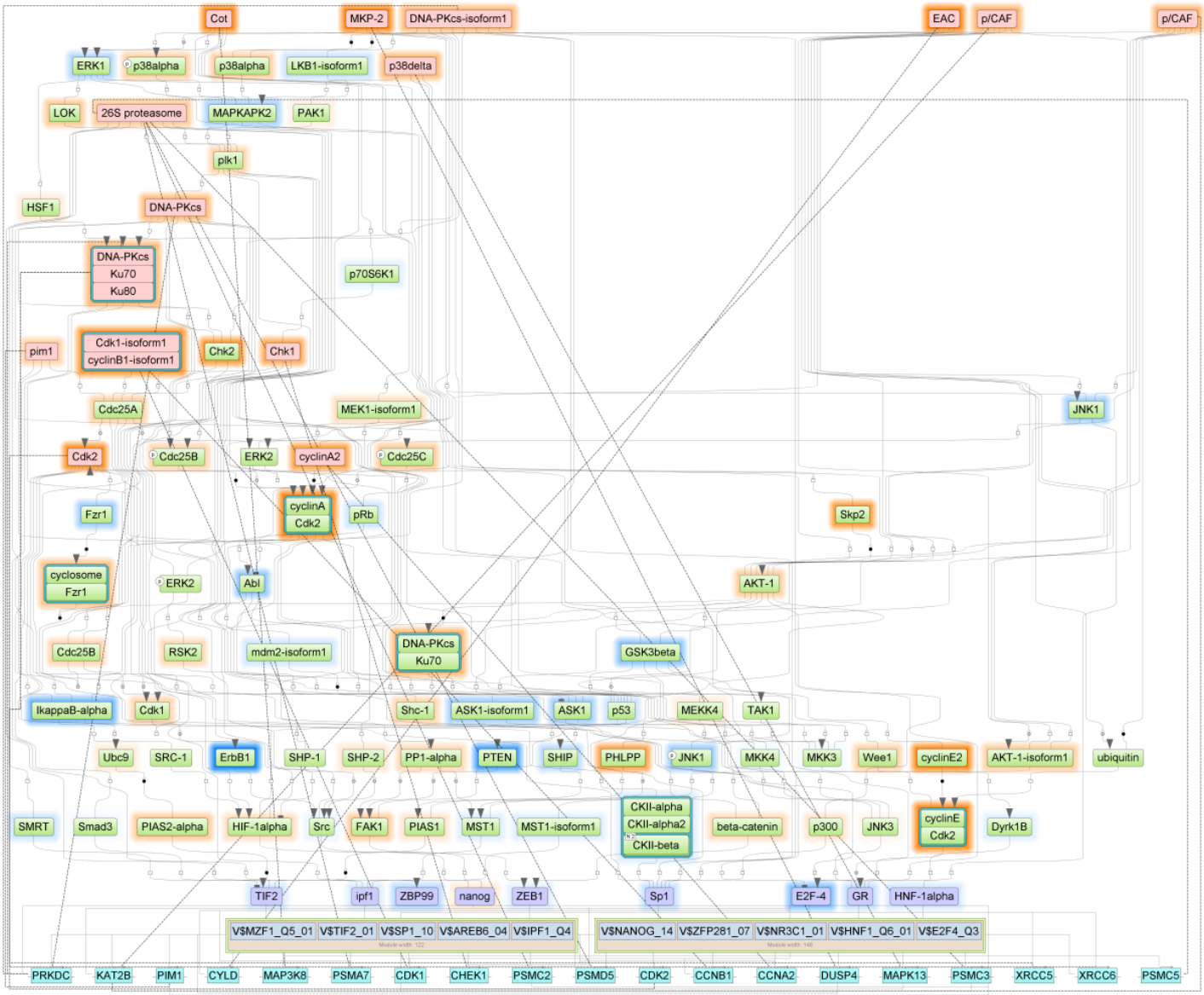


Figure 9. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp.

See full diagram →

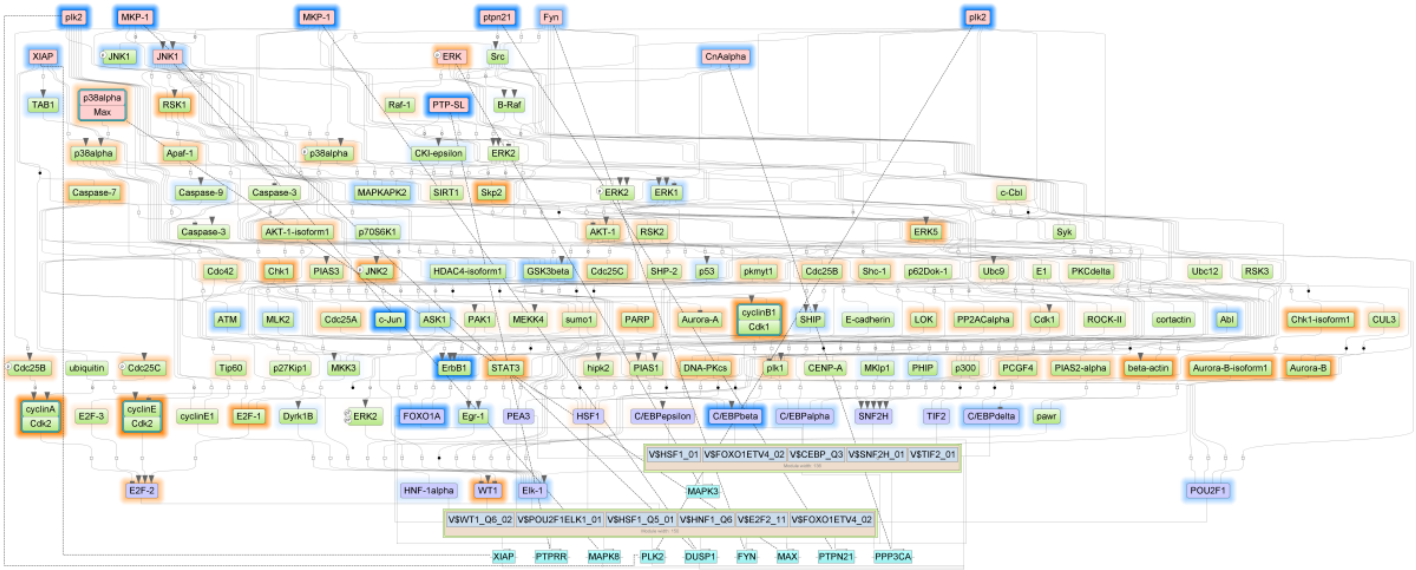


Figure 10. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp.

See full diagram →

4. Finding prospective drug targets

The identified master regulators that may govern pathology associated genes were checked for druggability potential using HumanPSD™ [5] database of gene-disease-drug assignments and PASS [11-13] software for prediction of biological activities of chemical compounds on the basis of a (Q)SAR approach. Respectively, for each master regulator protein we have computed two Druggability scores: HumanPSD Druggability score and PASS Druggability score. Where Druggability score represents the number of drugs that are potentially suitable for inhibition (or activation) of the corresponding target either according to the information extracted from medical literature (from HumanPSD™ database) or according to cheminformatics predictions of compounds activity against the examined target (from PASS software).

The cheminformatics druggability check is done using a pre-computed database of spectra of biological activities of chemical compounds from a library of all small molecular drugs from HumanPSD™ database, 2507 pharmaceutically active known chemical compounds in total. The spectra of biological activities has been computed using the program PASS [11-13] on the basis of a (Q)SAR approach.

If both Druggability scores were below defined thresholds (see Method section for the details) such master regulator proteins were not used in further analysis of drug prediction.

As a result we created the following two tables of prospective drug targets (top targets are shown here):

*Table 12. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score from HumanPSD™ database. **Druggability score** contains the number of drugs that are potentially suitable for inhibition (or activation) of the target. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
PSMA7	proteasome 20S subunit alpha 7	3	0.44	189
KAT2B	lysine acetyltransferase 2B	3	0.63	295
PDGFRA	platelet derived growth factor receptor alpha	8	2.93	393
PPP2CA	protein phosphatase 2 catalytic subunit alpha	3	0.71	412
PPP1CC	protein phosphatase 1 catalytic subunit gamma	4	0.44	469
PIM1	Pim-1 proto-oncogene, serine/threonine kinase	21	0.38	481

*Table 13. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score predicted by PASS software. Here, the **Druggability score** for master regulator proteins is computed as a sum of PASS calculated probabilities to be active as a target for various small molecular compounds. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.*

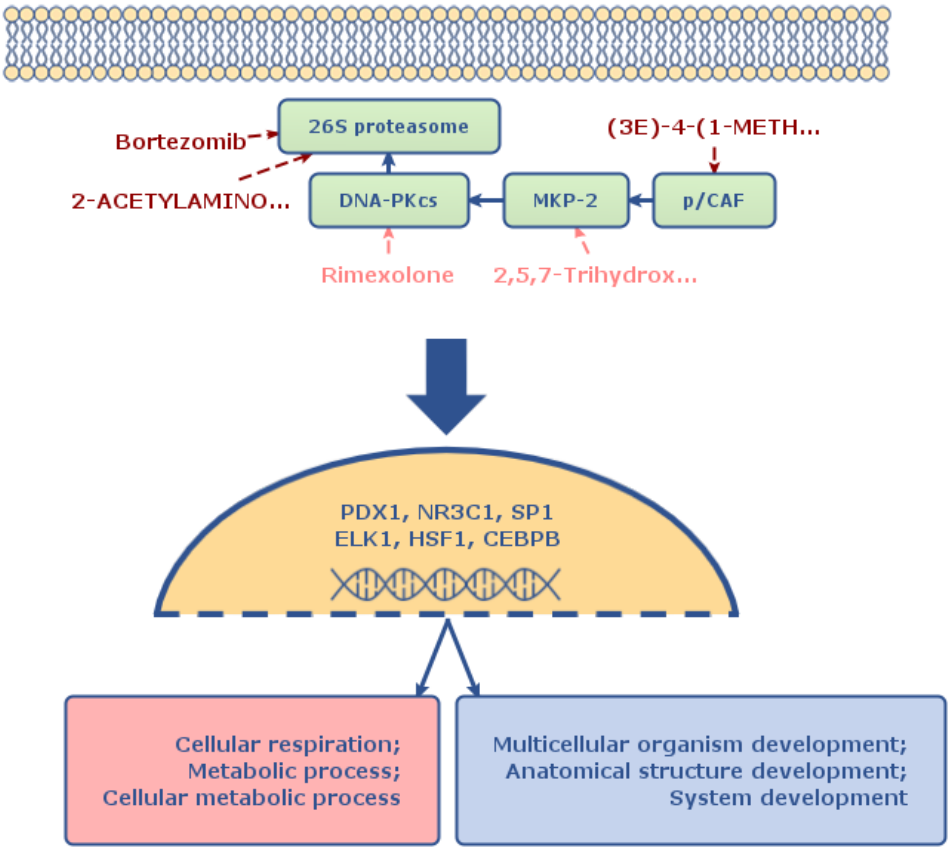
See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
DUSP4	dual specificity phosphatase 4	4.91	1.17	140
PSMC5	proteasome 26S subunit, ATPase 5	1.28	0.44	189
PSMD5	proteasome 26S subunit, non-ATPase 5	1.28	0.44	189
PSMA7	proteasome 20S subunit alpha 7	2.17	0.44	189
PSMC2	proteasome 26S subunit, ATPase 2	1.28	0.44	189
PSMC3	proteasome 26S subunit, ATPase 3	1.28	0.44	189

Below we represent schematically the main mechanism of the studied pathology. In the schema we considered the top two drug targets of each of the two categories computed above. In addition we have added two top identified master regulators for which no drugs may be identified yet, but that are playing the crucial role in the molecular mechanism of the studied pathology. Thus the molecular mechanism of the studied pathology was predicted to be mainly based on the following key master regulators:

- p/CAF
- DNA-PKcs
- MKP-2
- 26S proteasome

This result allows us to suggest the following schema of affecting the molecular mechanism of the studied pathology:



Drugs which are shown on this schema: 2,5,7-Trihydroxynaphthoquinone, Rimexolone, Bortezomib, (3E)-4-(1-METHYL-1H-INDOL-3-YL)BUT-3-EN-2-ONE and 2-ACETYLAMINO-4-METHYL-PENTANOIC ACID [1-(1-FORMYL-PENTYLCARBAMOYL)-3-METHYL-BUTYL]-AMIDE, should be considered as a prospective research initiative for further drug repurposing and drug development. These drugs were selected as top matching treatments to the most prospective drug targets of the studied pathology, however, these results should be considered with special caution and are to be used for research purposes only, as there is not enough clinical information for adapting these results towards immediate treatment of patients.
The drugs given in dark red color on the schema are FDA approved drugs or drugs which have gone through various phases of clinical trials as active treatments against the selected targets.
The drugs given in pink color on the schema are drugs, which were cheminformatically predicted to be active against the selected targets.

5. Identification of potential drugs

In the last step of the analysis we strived to identify known activities as well as drugs with cheminformatically predicted activities that are potentially suitable for inhibition (or activation) of the identified molecular targets in the context of specified human diseases(s).

Proposed drugs are top ranked drug candidates, that were found to be active on the identified targets and were selected from 4 categories:

1. FDA approved drugs or used in clinical trials drugs for the studied pathology;
2. Repurposing drugs used in clinical trials for other pathologies;
3. Drugs, predicted by PASS to be active against identified drug targets and against the studied pathology;
4. Drugs, predicted by PASS to be active against identified drug targets but for other pathologies.

Proposed drugs were selected on the basis of Drug rank which was computed from two scores:

- Target activity score (depends on ranks of all targets that were found for the selected drug);
- Disease activity score (weighted sum of number of clinical trials on disease(s) under study where the selected drug is known to be applied or PASS Disease activity score - cheminformatically predicted property of the compound to be active

against the studied disease(s)).

You can refer to the Methods section for more details on drug ranking procedure.

Top drugs of each category are given in the tables below:

Drugs approved in clinical trials

Table 14. FDA approved drugs or drugs used in clinical trials for the studied pathology (most promising treatment candidates selected for the identified drug targets on the basis of literature curation in HumanPSD™ database)
See full table →

Name	Target names	Drug rank	Disease activity score	Phase 4	Status (provided by Drugbank)
Pazopanib	ITK, PDGFRB, PDGFRA	48	7	Carcinoma, Renal Cell, Neoplasms, Noma	small molecule, approved
Palbociclib	CDK6, CDK4	78	1	Breast Neoplasms, Neoplasms	small molecule, approved
Imatinib	PDGFRB, PDGFRA	96	3	Breast Neoplasms, Gastrointestinal Stromal Tumors, Leukemia, Leukemia, Lymphoid, Leukemia, Myelogenous, Chronic, BCR-ABL Positive, Leukemia, Myeloid, Mastocytosis...	small molecule, approved
Bosutinib	CAMK2G, MAP2K1, CDK2	111	1	Leukemia, Myeloid	small molecule, approved
Regorafenib	PDGFRB, PDGFRA, RAF1	112	2	Colorectal Neoplasms, Gastrointestinal Stromal Tumors, Neoplasms, Rectal Neoplasms	small molecule, approved

Repurposing drugs

Table 15. Repurposed drugs used in clinical trials for other pathologies (prospective drugs against the identified drug targets on the basis of literature curation in HumanPSD™ database)
See full table →

Name	Target names	Drug rank	Phase 4	Status (provided by Drugbank)
Minocycline	CASP3, CASP1, CYCS	110	Acne Vulgaris, Affect, Alopecia, Autistic Disorder, Bacterial Infections, Bipolar Disorder, Chronic Periodontitis...	small molecule, approved, investigational
Trastuzumab	FCGR2A, ERBB2, FCGR1A	122	Breast Neoplasms, Neoplasms, Stomach Neoplasms	biotech, approved, investigational
Vitamin E	PPP2CB, PPP2CA	127	Angina Pectoris, Variant, Asphyxia, Cicatrix, Cicatrix, Hypertrophic, Diabetes Mellitus, Dyslipidemias, Epilepsy...	small molecule, approved, nutraceutical
Tofacitinib	JAK3, JAK2, JAK1	128	Arthritis, Arthritis, Rheumatoid	small molecule, approved
Fica	CASP7	129	Acute Coronary Syndrome, Arteriosclerosis, Coronary Artery Disease, HIV Infections, Hyperlipidemias, Hypertriglyceridemia, Infection...	small molecule, experimental

No prospective drugs were found, which would be predicted by PASS software to be active against the identified drug targets and would be predicted to have biological activity against the studied disease(s).

Table 16. Prospective drugs, predicted by PASS software to be active against the identified drug targets, though without cheminformatically predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)
See full table →

Name	Target names	Drug rank	Target activity score
2,5,7-Trihydroxynaphthoquinone	MAPK10, MAPK1, DUSP23, MAPK9, MAPK4, MAPK6, PTPRC...	26	1.55
3,5-Diaminophthalhydrazide	RPS6KA3, IRAK4, CAMK2G, RPS6KA2, CSNK1A1, PRKD3, CSNK1G2...	32	1.63
6-Nitroindazole	RPS6KA3, CAMK2G, CDK6, IRAK4, CSNK1A1, PRKACA, EPHA4...	34	4.51
2,6-Dihydroanthra/1,9-Cd/Pyrazol-6-One	MAPK10, RPS6KA3, IRAK4, CDK6, CAMK2G, CSNK1A1, PAK2...	38	5.43
7-[4-(Dimethylamino)Phenyl]-N-Hydroxy-4,6-Dimethyl-7-Oxo-2,4-Heptadienamide	HDAC3, HDAC1	44	0.74

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Pazopanib, Minocycline and 2,5,7-Trihydroxynaphthoquinone. These drugs were selected for acting on the following targets: PDGFRA, CASP1 and DUSP4, which were predicted to be active in the molecular mechanism of the studied pathology.

The selected drugs are top ranked drug candidates from each of the four categories of drugs: (1) FDA approved drugs or used in clinical trials drugs for the studied pathology; (2) repurposing drugs used in clinical trials for other pathologies; (3) drugs, predicted by PASS software to be active against the studied pathology; (4) drugs, predicted by PASS software to be repurposed from other pathologies.

6. Conclusion

We applied the software package "Genome Enhancer" to a multi-omics data set that contains *transcriptomics and epigenomics* data. The study is done in the context of *Ovarian Neoplasms*. The data were pre-processed, statistically analyzed and differentially expressed genes were identified. Also checked was the enrichment of GO or disease categories among the studied gene sets.

We propose the following drugs as most promising candidates for treating the pathology under study:

Pazopanib, Minocycline and 2,5,7-Trihydroxynaphthoquinone

These drugs were selected for acting on the following targets: PDGFRA, CASP1 and DUSP4, which were predicted to be involved in the molecular mechanism of the pathology under study.

The identified molecular mechanism of the studied pathology was predicted to be mainly based on the following key drug targets:

p/CAF, DNA-PKcs, MKP-2 and 26S proteasome

These potential drug targets should be considered as a prospective research initiative for further drug repurposing and drug development purposes. The following drugs were predicted as, matching those drug targets: 2,5,7-Trihydroxynaphthoquinone, Rimexolone, Bortezomib, (3E)-4-(1-METHYL-1H-INDOL-3-YL)BUT-3-EN-2-ONE and 2-ACETYLAMINO-4-METHYL-PENTANOIC ACID [1-(1-FORMYL-PENTYLCARBAMOYL)-3-METHYL-BUTYL]-AMIDE. These drugs should be considered with special caution for research purposes only.

In this study, we came up with a detailed signal transduction network regulating differentially expressed genes in the studied pathology. In this network we have revealed the following top master regulators (signaling proteins and their complexes) that play a crucial role in the molecular mechanism of the studied pathology, which can be proposed as the most promising molecular targets for further drug repurposing and drug development initiatives.

- p/CAF
- DNA-PKcs
- MKP-2
- 26S proteasome

Potential drug compounds which can be affecting these targets can be found in the "Finding prospective drug targets" section.

7. Methods

Databases used in the study

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs described in the TRANSFAC® library, release 2021.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2021.1 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transpath). A comprehensive signal transduction network of human cells is built by the software on the basis of reactions annotated in TRANSPATH®.
The information about drugs corresponding to identified drug targets and clinical trials references were extracted from HumanPSD™ database, release 2021.1 (https://genexplain.com/humanpsd).
The Ensembl database release Human100.38 (hg38) (http://www.ensembl.org) was used for gene IDs representation and Gene Ontology (GO) (http://geneontology.org) was used for functional classification of the studied gene set.

Methods for the analysis of enriched transcription factor binding sites and composite modules

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs. The motifs are specified using position weight matrices (PWMs) that give weights to each nucleotide in each position of the DNA binding motif for a transcription factor or a group of them.
We search for transcription factor binding sites (TFBS) that are enriched in the promoters and enhancers under study as compared to a background sequence set such as promoters of genes that were not differentially regulated under the condition of the experiment. We denote study and background sets briefly as Yes and No sets. In the current work we used a workflow

considering promoter sequences of a standard length of 1100 bp (-1000 to +100). The error rate in this part of the pipeline is controlled by estimating the adjusted p-value (using the Benjamini-Hochberg procedure) in comparison to the TFBS frequency found in randomly selected regions of the human genome (adj.p-value < 0.01).
We have applied the CMA algorithm (Composite Module Analyst) for searching composite modules [7] in the promoters and enhancers of the Yes and No sets. We searched for a composite module consisting of a cluster of 10 TFs in a sliding window of 200-300 bp that statistically significantly separates sequences in the Yes and No sets (minimizing Wilcoxon p-value).

Methods for finding master regulators in networks

We searched for master regulator molecules in signal transduction pathways upstream of the identified transcription factors. The master regulator search uses a comprehensive signal transduction network of human cells. The main algorithm of the master regulator search has been described earlier [3,4]. The goal of the algorithm is to find nodes in the global signal transduction network that may potentially regulate the activity of a set of transcription factors found at the previous step of the analysis. Such nodes are considered as most promising drug targets, since any influence on such a node may switch the transcriptional programs of hundreds of genes that are regulated by the respective TFs. In our analysis, we have run the algorithm with a maximum radius of 12 steps upstream of each TF in the input set. The error rate of this algorithm is controlled by applying it 10000 times to randomly generated sets of input transcription factors of the same set-size. Z-score and FDR value of ranks are calculated then for each potential master regulator node on the basis of such random runs (see detailed description in [9]). We control the error rate by the FDR threshold 0.05.

Methods for analysis of pharmaceutical compounds

We seek for the optimal combination of molecular targets (key elements of the regulatory network of the cell) that potentially interact with pharmaceutical compounds from a library of known drugs and biologically active chemical compounds, using information about known drugs from HumanPSD™ and predicting potential drugs using PASS program.

Method for analysis of known pharmaceutical compounds

We selected compounds from HumanPSD™ database that have at least one target. Next, we sort compounds using "*Drug rank*" that is sum of two other ranks:

1. ranking by "Target activity score" (*T-score*$_{PSD}$),
2. ranking by "Disease activity score" (*D-score*$_{PSD}$).

"Target activity score" (*T-score*$_{PSD}$) is calculated as follows:

$$T\text{-}score_{PSD} = -\frac{|T|}{|T| + w(|AT| - |T|))} \sum_{t \in T} log_{10}\left(\frac{rank(t)}{1 + maxRank(T)}\right),$$

where *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier, *rank(t)* is rank of given target, *maxRank(T)* equals *max(rank(t))* for all targets *t* in *T*.
We use following formula to calculate "Disease activity score" (*D-score*$_{PSD}$):

$$D\text{-}score_{PSD} = \begin{cases} \sum_{d \in D} \sum_{p \in P} phase(d,p) \\ 0, \ D = \varnothing \end{cases},$$

where *D* is the set of selected diseases, and if *D* is empty set, *D-score*$_{PSD}$*=0*. *P* is a set of all known phases for each disease, *phase(p,d)* equals to the phase number if there are known clinical trials for the selected disease on this phase and zero otherwise.

Method for prediction of pharmaceutical compounds

In this study, the focus was put on compounds with high pharmacological efficiency and low toxicity. For this purpose, comprehensive library of chemical compounds and drugs was subjected to a SAR/QSAR analysis. This library contains 13040 compounds along with their pre-calculated potential pharmacological activities of those substances, their possible side and toxic effects, as well as the possible mechanisms of action. All biological activities are expressed as probability values for a substance to exert this activity (*Pa*).
We selected compounds that satisfied the following conditions:

1. Toxicity below a chosen toxicity threshold (defines as *Pa*, probability to be active as toxic substance).
2. For all predicted pharmacological effects that correspond to a set of user selected disease(s) *Pa* is greater than a chosen effect threshold.
3. There are at least 2 targets (corresponding to the predicted activity-mechanisms) with predicted *Pa* greater than a chosen target threshold.

The maximum *Pa* value for all toxicities corresponding to the given compound is selected as the "Toxicity score". The maximum *Pa* value for all activities corresponding to the selected diseases for the given compound is used as the "Disease activity score".
"Target activity score" (T-score) is calculated as follows:

$$T\text{-}score(s) = \frac{|T|}{|T| + w(|AT| - |T|))} \sum_{m \in M(s)} \left(pa(m) \sum_{g \in G(m)} IAP(g) optWeight(g) \right),$$

where *M(s)* is the set of activity-mechanisms for the given structure (which passed the chosen threshold for activity-mechanisms *Pa*); *G(m)* is the set of targets (converted to genes) that corresponds to the given activity-mechanism (m) for the given compound; *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for gene from *G(m)*; *optWeight(g)* is the additional weight multiplier for gene. *T* is set of all targets related to the compound intersected with input list, |*T*| is number of elements in *T*, *AT* and |*AT*| are set set of all targets related to the compound and number of elements in it, *w* is weight multiplier.
"Druggability score" (D-score) is calculated as follows:

$$D\text{-}score(g) = IAP(g) \sum_{s \in S(g)} \sum_{m \in M(s,g)} pa(m),$$

where *S(g)* is the set of structures for which target list contains given target, *M(s,g)* is the set of activity-mechanisms (for the given structure) that corresponds to the given gene, *pa(m)* is the probability to be active of the activity-mechanism (m), *IAP(g)* is the invariant accuracy of prediction for the given gene.

8. References

1. Kel A, Voss N, Jauregui R, Kel-Margoulis O, Wingender E. Beyond microarrays: Finding key transcription factors controlling signal transduction pathways. *BMC Bioinformatics.* **2006**;7(S2), S13. doi:10.1186/1471-2105-7-s2-s13
2. Stegmaier P, Voss N, Meier T, Kel A, Wingender E, Borlak J. Advanced Computational Biology Methods Identify Molecular Switches for Malignancy in an EGF Mouse Model of Liver Cancer. *PLoS ONE.* **2011**;6(3):e17738. doi:10.1371/journal.pone.0017738
3. Koschmann J, Bhar A, Stegmaier P, Kel A, Wingender E. "Upstream Analysis": An Integrated Promoter-Pathway Analysis Approach to Causal Interpretation of Microarray Data. *Microarrays.* **2015**;4(2):270-286. doi:10.3390/microarrays4020270.
4. Kel A, Stegmaier P, Valeev T, Koschmann J, Poroikov V, Kel-Margoulis OV, and Wingender E. Multi-omics "upstream analysis" of regulatory genomic regions helps identifying targets against methotrexate resistance of colon cancer. *EuPA Open Proteom.* **2016**;13:1-13. doi:10.1016/j.euprot.2016.09.002
5. Michael H, Hogan J, Kel A et al. Building a knowledge base for systems pathology. *Brief Bioinformatics.* **2008**;9(6):518-531. doi:10.1093/bib/bbn038
6. Matys V, Kel-Margoulis OV, Fricke E, Liebich I, Land S, Barre-Dirrie A, Reuter I, Chekmenev D, Krull M, Hornischer K, Voss N, Stegmaier P, Lewicki-Potapov B, Saxel H, Kel AE, Wingender E. TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res.* **2006**;34(90001):D108-D110. doi:10.1093/nar/gkj143
7. Kel AE, Gössling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. MATCH: A tool for searching transcription factor binding sites in DNA sequences. *Nucleic Acids Res.* **2003**;31(13):3576-3579. doi:10.1093/nar/gkg585
8. Waleev T, Shtokalo D, Konovalova T, Voss N, Cheremushkin E, Stegmaier P, Kel-Margoulis O, Wingender E, Kel A. Composite Module Analyst: identification of transcription factor binding site combinations using genetic algorithm. *Nucleic Acids Res.* **2006**;34(Web Server issue):W541-5.
9. Krull M, Pistor S, Voss N, Kel A, Reuter I, Kronenberg D, Michael H, Schwarzer K, Potapov A, Choi C, Kel-Margoulis O, Wingender E. TRANSPATH: an information resource for storing and visualizing signaling pathways and their pathological aberrations. *Nucleic Acids Res.* **2006**;34(90001):D546-D551. doi:10.1093/nar/gkj107
0. Boyarskikh U, Pintus S, Mandrik N, Stelmashenko D, Kiselev I, Evshin I, Sharipov R, Stegmaier P, Kolpakov F, Filipenko M, Kel A. Computational master-regulator search reveals mTOR and PI3K pathways responsible for low sensitivity of NCI-H292 and A427 lung cancer cell lines to cytotoxic action of p53 activator Nutlin-3. *BMC Med Genomics.* **2018**;11(1):12. doi:10.1186/1471-2105-7-s2-s13
1. Filimonov D, Poroikov V. Probabilistic Approaches in Activity Prediction. Varnek A, Tropsha A. *Cheminformatics Approaches to Virtual Screening.* Cambridge (UK): RSC Publishing. **2008**;:182-216.
2. Filimonov DA, Poroikov VV. Prognosis of specters of biological activity of organic molecules. *Russian chemical journal.* **2006**;50(2):66-75 (russ)
3. Filimonov D, Poroikov V, Borodina Y, Gloriozova T. Chemical Similarity Assessment Through Multilevel Neighborhoods of Atoms: Definition and Comparison with the Other Descriptors. *ChemInform.* **1999**;39(4):666-670. doi:10.1002/chin.199940210

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Supplementary material

1. Supplementary table 1 - Up-regulated genes
2. Supplementary table 2 - Down-regulated genes
3. Supplementary table 3 - Detailed report. Composite modules and master regulators (up-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in Experiment: cisplatin-resistant vs. Control: cisplatin-sensitive).
5. Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.

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