CCND1 and DUSP9 are promising druggable targets for treating Neoplasm Metastasis and Osteosarcoma that control activity of EGR1, EP300 and RXRA transcription factors on promoters of differentially expressed genes

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Genome Enhancer release 2.0 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2020.2)

Abstract

In the present study we applied the software package "Genome Enhancer" to a multiomics data set that contains *transcriptomics and proteomics* data. The study is done in the context of *Neoplasm Metastasis and Osteosarcoma*. 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: EGR1, EP300, NR3C1, RXRA, SMAD2 and SMAD1. The subsequent network analysis suggested

- integrins
- SIRT1
- Cdk4-isoform1:cyclinD1a
- TFIIH-CAK
- MKP-4

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: Bosutinib, Ingenol Mebutate, D-Myo-Inositol-Hexasulphate and 3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,2-.C.]Pyrazole.

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 2507 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
Proteomics	Proteomics
RNAseq	Transcriptomics

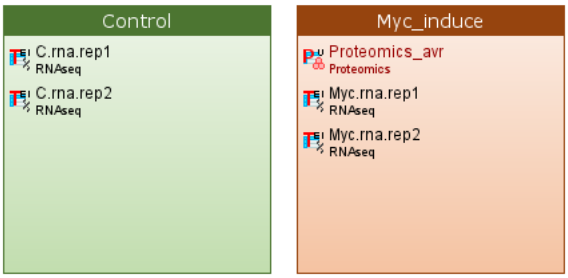


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

3. Results

We have compared the following conditions: Myc_induce *versus* Control.

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: "Myc_induce" with "Control". 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 5047 upregulated genes (LogFC>0) out of which 1195 genes were found as significantly upregulated (p-value<0.1) and 4524 downregulated genes (LogFC<0) out of which 1169 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 Myc_induce vs. Control.*
See full table →

ID	Gene symbol	Gene description	logFC	P.Value	adj.P.Val
ENSG00000136997	MYC	MYC proto-oncogene, bHLH transcription factor	5.96	7.45E-6	7.13E-2
ENSG00000164076	CAMKV	CaM kinase like vesicle associated	4.08	8.1E-5	0.13
ENSG00000120738	EGR1	early growth response 1	3.51	5.46E-4	0.14
ENSG00000173110	HSPA6	heat shock protein family A (Hsp70) member 6	3.14	1.66E-4	0.13
ENSG00000123360	PDE1B	phosphodiesterase 1B	2.85	1.08E-4	0.13
ENSG00000137571	SLCO5A1	solute carrier organic anion transporter family member 5A1	2.79	9.53E-5	0.13
ENSG00000078549	ADCYAP1R1	ADCYAP receptor type I	2.69	2.44E-3	0.14
ENSG00000143333	RGS16	regulator of G protein signaling 16	2.69	2.47E-4	0.13
ENSG00000170345	FOS	Fos proto-oncogene, AP-1 transcription factor subunit	2.57	4.12E-3	0.15
ENSG00000117322	CR2	complement C3d receptor 2	2.46	2.57E-4	0.13

*Table 3. Top ten significant **down-regulated** genes in Myc_induce vs. Control.*
See full table →

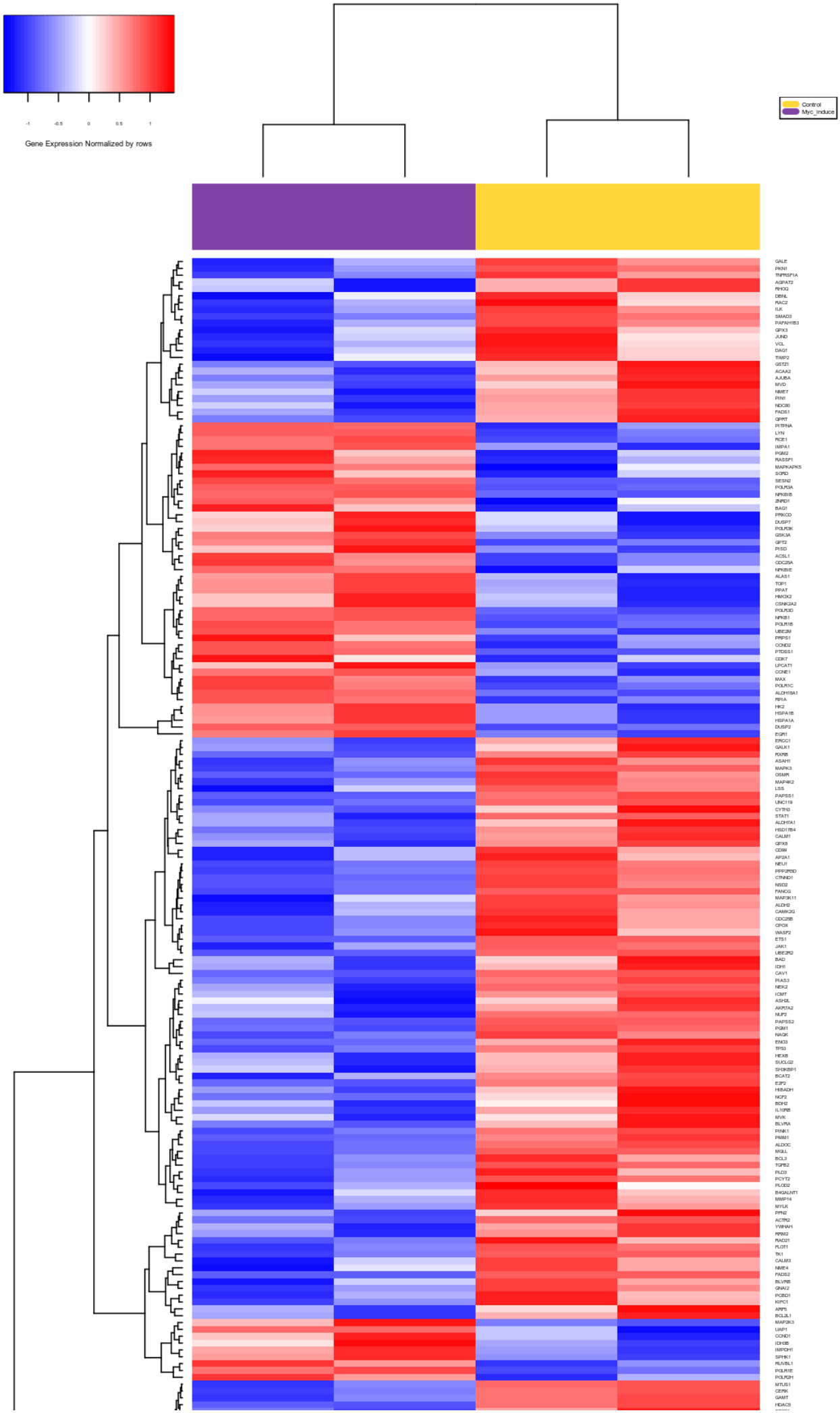
ID	Gene symbol	Gene description	logFC	P.Value	adj.P.Val
ENSG00000116774	OLFML3	olfactomedin like 3	-3.06	1.11E-4	0.13
ENSG00000138131	LOXL4	lysyl oxidase like 4	-2.62	8.88E-4	0.14
ENSG00000187867	PALM3	paralemmin 3	-2.62	2.65E-3	0.14
ENSG00000205542	TMSB4X	thymosin beta 4 X-linked	-2.58	2.22E-4	0.13
ENSG00000158825	CDA	cytidine deaminase	-2.54	3.49E-4	0.13
ENSG00000127129	EDN2	endothelin 2	-2.49	3.28E-4	0.13
ENSG00000182667	NTM	neurotrimin	-2.48	4.08E-4	0.13
ENSG00000114115	RBP1	retinol binding protein 1	-2.46	1.06E-4	0.13
ENSG00000132746	ALDH3B2	aldehyde dehydrogenase 3 family member B2	-2.35	1.93E-4	0.13
ENSG00000188042	ARL4C	ADP ribosylation factor like GTPase 4C	-2.29	1.87E-3	0.14

3.2. 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 Myc_induce vs. Control

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


-1
-0.5
0
0.5
1
Gene Expression Normalized by rows
Control
Myc_Induce
GALE
PKN1
TNFRSF1A
AGPAT2
RHOQ
DBNL
RAC2
ILK
SMAD3
PAPAH1B3
GPX3
JUND
VCL
DAG1
TIMP2
GSTZ1
ACAA2
AJUBA
MVD
NMET
PIN1
NDC80
FADS1
GPRT
PITPNA
LYN
RCE1
IMPA1
PGM2
RASSF1
MAPKAPK5
SORD
SESN2
PGLYRA
NPKB1B
ZNRD1
BAG1
PRKCD
DUSP7
PGLYR3K
GSK3A
GPT2
PISD
ACSL1
CDC25A
NPKB1E
ALAS1
TOP1
PRAT
HMGX2
CSNK2A2
PGLYR3D
NPKB1
PGLYR1B
UBE2M
PRPS1
COND2
PTDSS1
CDK7
LPCAT1
CCNE1
MAX
PGLYR1C
ALDH18A1
RPIA
HK2
HSPA1B
HSPA1A
DUSP2
EGR1
ERCC1
GALK1
PXRB
ASAH1
MAPK3
OSMR
MAPHK2
LSS
PAPSS1
UNC119
CYTH3
STAT1
ALDHTA1
HSD17B4
CALM1
GPX8
CD99
AP2A1
NEU1
PPP2R5D
CTNND1
NSD2
FANCG
MAP3K11
ALDH2
CAMK2G
CDC25B
CPOX
WASF2
ETS1
JAK1
UBE2K2
BAD
IDH1
CAV1
PIAS3
NEK2
ICMT
ASH3L
AKR7A2
NUP3
PAPSS2
PGM1
NAGK
ENO3
TP53
HEXB
SUCLG2
SH3KBP1
BCAT2
E2F2
HIBADH
NCP2
BDH2
IL10RB
MVK
BLVRA
PINK1
PNMT
ALDOC
MGLL
BCL3
TGFB2
PLD3
PCYT2
PLOD2
B4GALNT1
MMP14
MYLK
PPN2
ACTR2
YWHAH
RPN2
RAD21
PLOT1
TK1
CALM3
NME4
FADS2
BLVRB
GNAI2
PCBD1
KIFC1
AP5S
BCL2L1
MAP2K3
UAP1
CCND1
IDH3B
IMPDH1
SPHK1
RUVBL1
PGLYR1E
POLR2H
MTUS1
CERK
GAMT
HDAC5

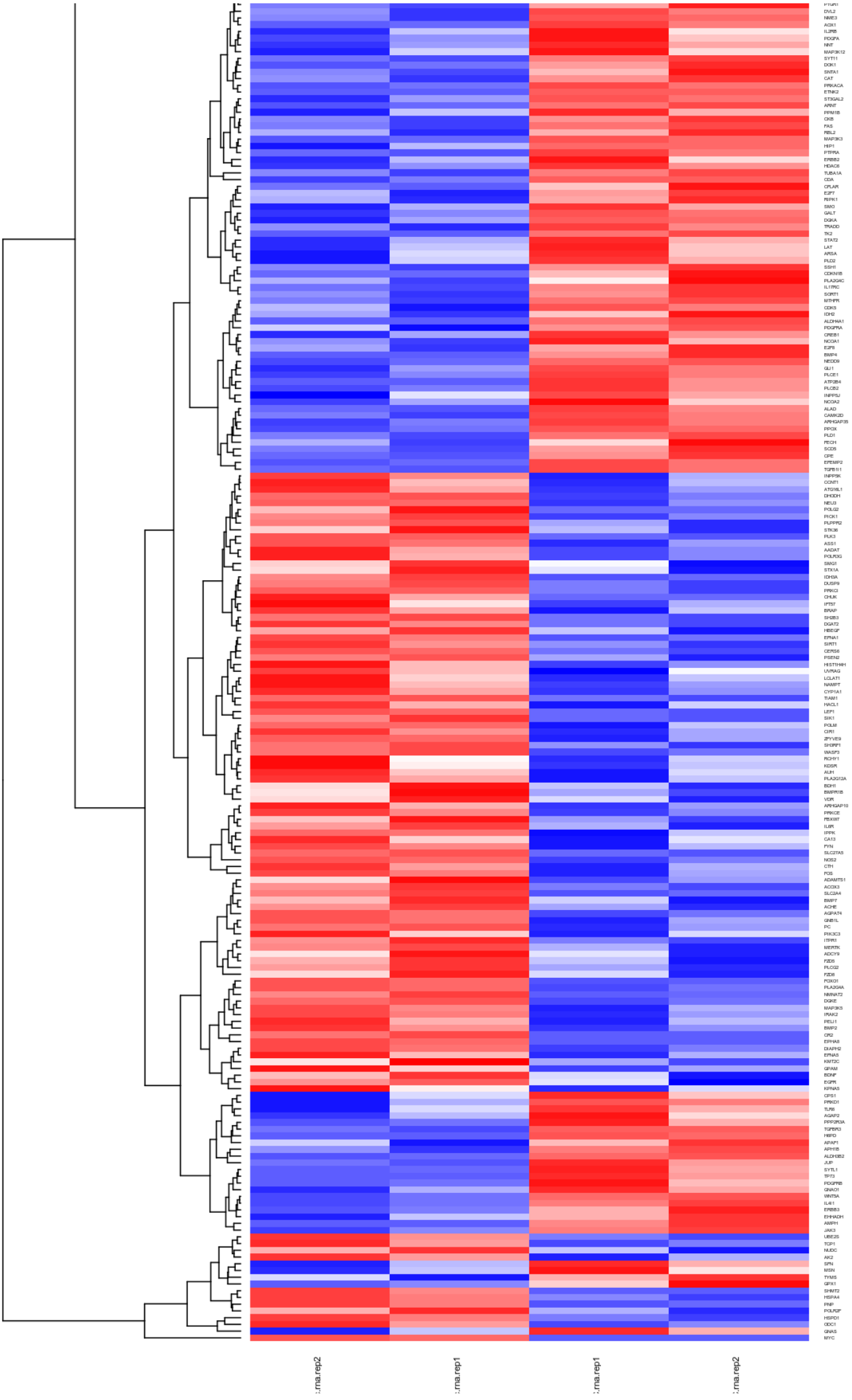

DVL2
NME3
AGK1
IL2RB
PDGFA
NNT
MAP3K12
SYT11
DOK1
SNTA1
CAT
PRKACA
ETNK2
ST3GAL2
ARNT
PRNT1B
GKB
FAS
RBL2
MAP3K3
HIP1
PTPRA
ERBB2
HDAC6
TUBA1A
CDA
CPLANI
E2F7
RIPK1
SMO
GALT
DGKA
TRADD
TK2
STAT2
LAT
ARSA
PLD2
SSH1
CDKN1B
PLA2G4C
IL17RC
SORT1
MTHFR
CDK9
IDH2
ALDH4A1
PDGFRA
CREB1
NCOA1
E2F8
BMPR1
NEDD9
GLI1
PLCE1
ATP2B4
PLCB2
INPP5J
NCOA2
ALAD
CAMK2D
ARHGAP35
PROX
PLD1
FECH
SCD5
CPE
EFEMP2
TGFB1I1
INPP5K
CCNT1
ATG16L1
DHODH
NEU3
POLG2
PICK1
PLPPR2
STK36
PLK3
ASS1
AADAT
POLR3G
SNG1
STX1A
IDH3A
DUSP9
PRKCI
CHUK
IPT57
BRAP
SH2B3
DGAT2
HBEGF
EPHA1
SIRT1
CERS6
PSEN2
HIST1H1H
UVRAG
LCLAT1
NAMPT
CYP1A1
TIAM1
HACL1
LEF1
SIK1
POLM
CIP1
ZFYVE9
SH3PF1
WASF3
RCHY1
KDSR
AUH
PLA2G12A
BDH1
BMPR1B
VDR
ARHGAP10
PRKCE
RBX1W7
IL6R
IPPK
CA13
FYN
SLC27A5
NOS2
CTH
POS
ADAMTS1
ACOX3
SLC2A4
BMP7
ACHE
AGPAT4
GNB1L
PC
PIK3C3
ITPR1
MERTK
ADCY9
FZD5
PLCG2
FZD6
FOXO1
PLA2G4A
NMNAT2
DGKE
MAP3K5
IPMK2
PELI1
BMP2
CR2
EPHA8
DIAPH2
EPHA5
KNTC
GFAM
BDNF
EGFR
KIFAP3
CPS1
PRKD1
TLR6
AGAP2
PPP2R3A
TGFBR3
HSPD
AFAP1
APH1B
ALDH3B2
JUP
SYTL1
TPT3
PDGFRB
GNAO1
WNT5A
IL4I1
ERBB3
EHHADH
AMPH
JAK3
UBE2S
TOP1
NUDC
AK2
SPN
MSN
TYMS
GFX1
SHMT2
HSPA4
PNP
POLR2F
HSPD1
ODC1
GNAS
MYC
:.rna.rep2
:.rna.rep1
:.rna.rep1
:.rna.rep2

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 Myc_induce vs. Control:

1195 significant up-regulated genes were taken for the mapping.

GO (biological process)

Figure 3. Enriched GO (biological process) of up-regulated genes in Myc_induce vs. Control.
Full classification →

TRANSPATH® Pathways (2020.2)

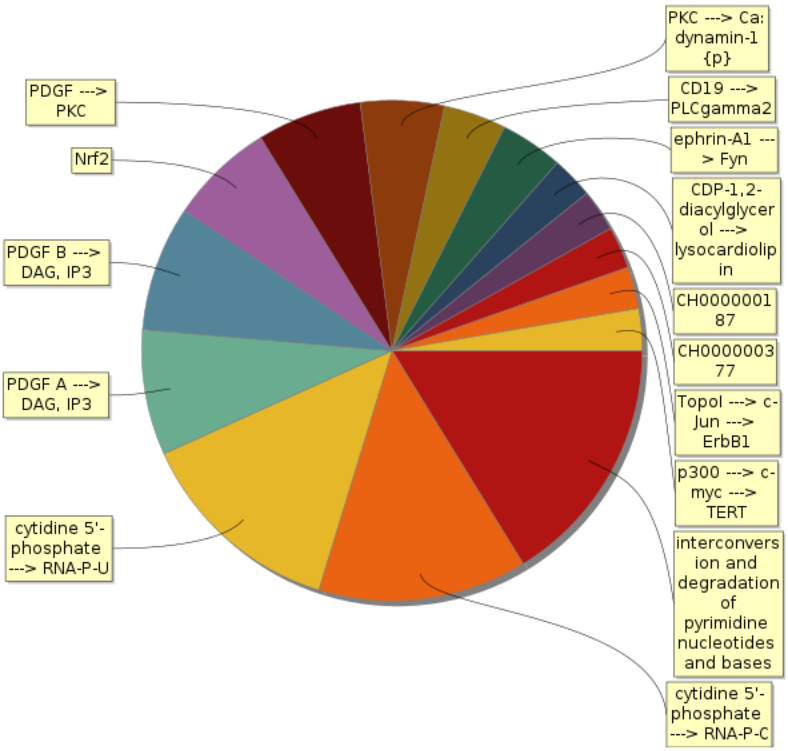


Figure 4. Enriched TRANSPATH® Pathways (2020.2) of up-regulated genes in Myc_induce vs. Control.
Full classification →

HumanPSD(TM) disease (2020.2)

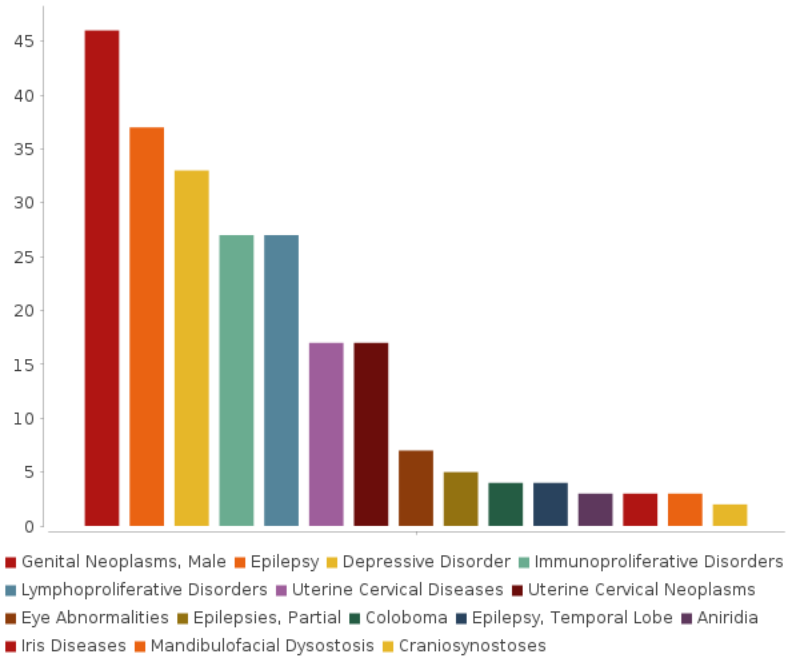


Figure 5. Enriched HumanPSD(TM) disease (2020.2) of up-regulated genes in Myc_induce vs. Control. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.
Full classification →

Down-regulated genes in Myc_induce vs. Control:

1169 significant down-regulated genes were taken for the mapping.

GO (biological process)

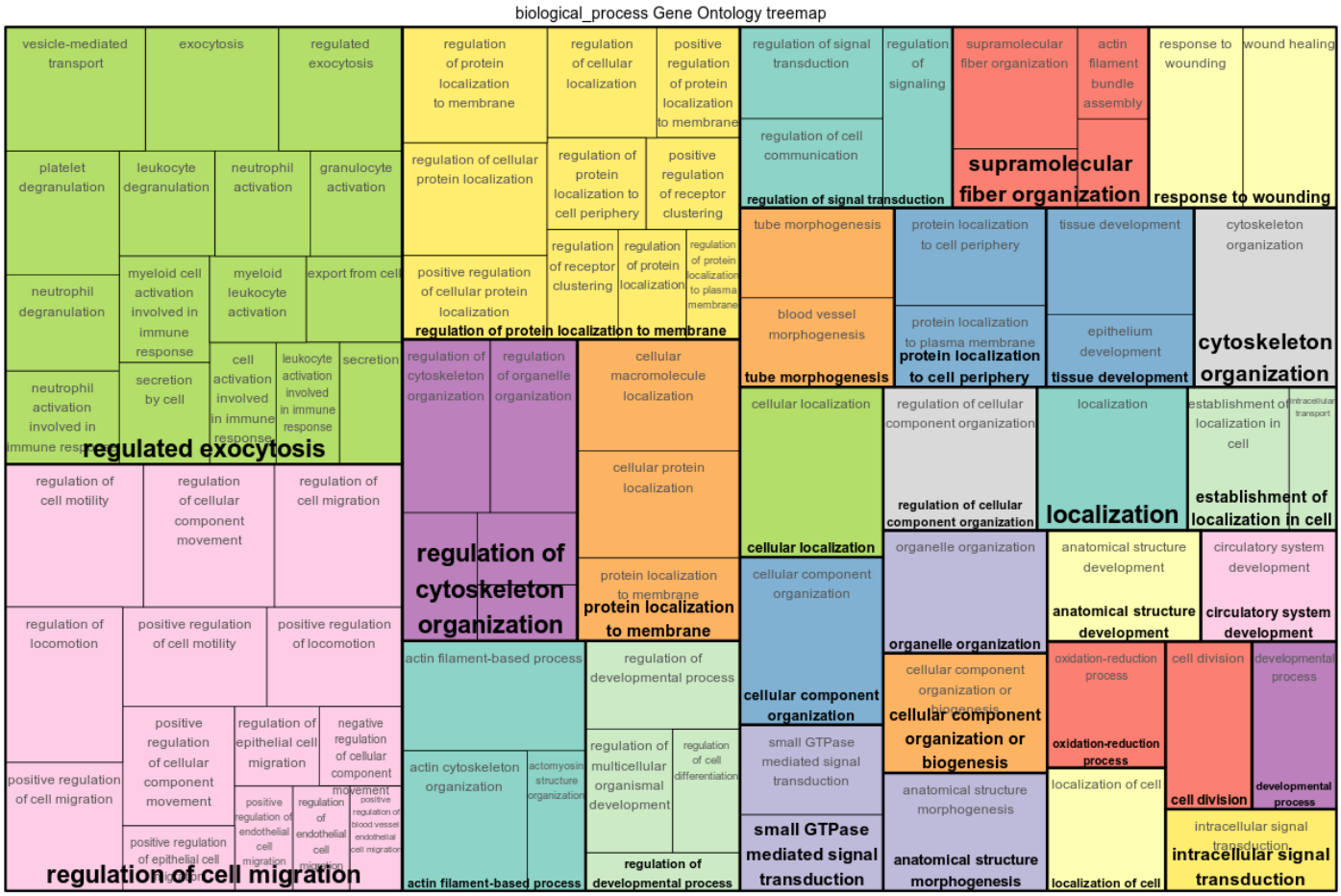


Figure 6. Enriched GO (biological process) of down-regulated genes in Myc_induce vs. Control.
Full classification →

TRANSPATH® Pathways (2020.2)

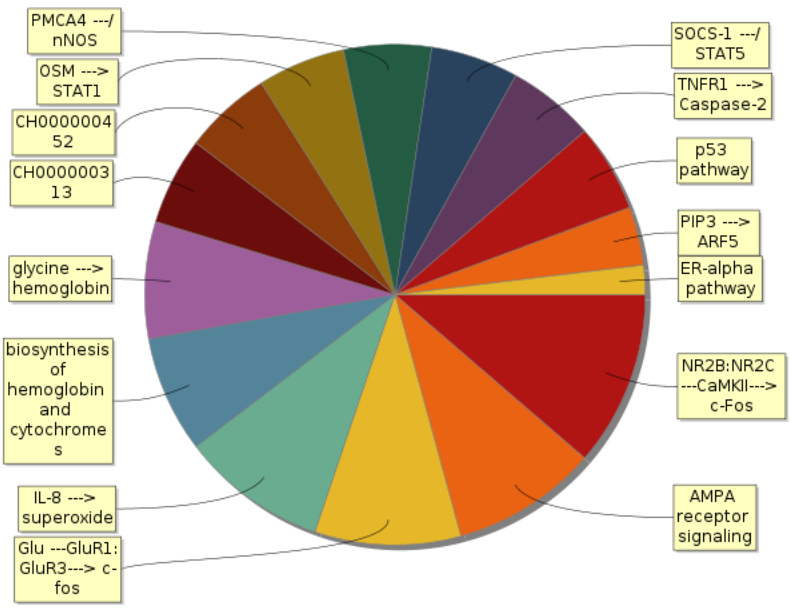


Figure 7. Enriched TRANSPATH® Pathways (2020.2) of down-regulated genes in Myc_induce vs. Control.
Full classification →

HumanPSD(TM) disease (2020.2)

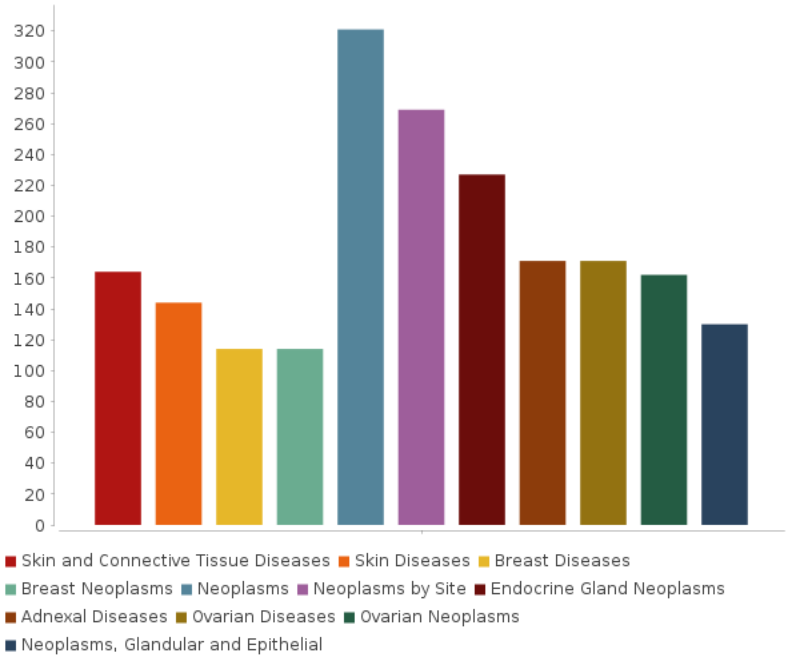


Figure 8. Enriched HumanPSD(TM) disease (2020.2) of down-regulated genes in Myc_induce vs. Control. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.

Full classification →

3.3. Identification of proteins

In the first step of the proteome data analysis target proteins were identified from the uploaded experimental data (the list of 4665 proteins) and were converted to corresponding genes. These genes were used in the further steps of analysis.

Table 4. Top ten the list of genes provided as input in Myc_induce.

See full table →

ID	Gene description	Gene symbol	Proteomics_avr
ENSG00000173598	nudix hydrolase 4	NUDT4	4.36
ENSG00000100335	mitochondrial elongation factor 1	MIEF1	3.8
ENSG00000115884	syndecan 1	SDC1	3.62
ENSG00000102910	lon peptidase 2, peroxisomal	LONP2	3.3
ENSG00000179046	tripartite motif family like 2	TRIML2	2.87
ENSG00000114648	kelch like family member 18	KLHL18	2.76
ENSG00000170525	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	PFKFB3	2.69
ENSG00000120949	TNF receptor superfamily member 8	TNFRSF8	2.46
ENSG00000188158	NHS actin remodeling regulator	NHS	2.46
ENSG00000119599	DDB1 and CUL4 associated factor 4	DCAF4	2.42

3.4. Functional classification of expressed proteins

A functional analysis of expressed proteins was done by mapping the protein IDs 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 9-11 show the most significant categories.

The list of proteins provided as input in Myc_induce:

4660 the list of genes provided as input genes were taken for the mapping.

GO (biological process)

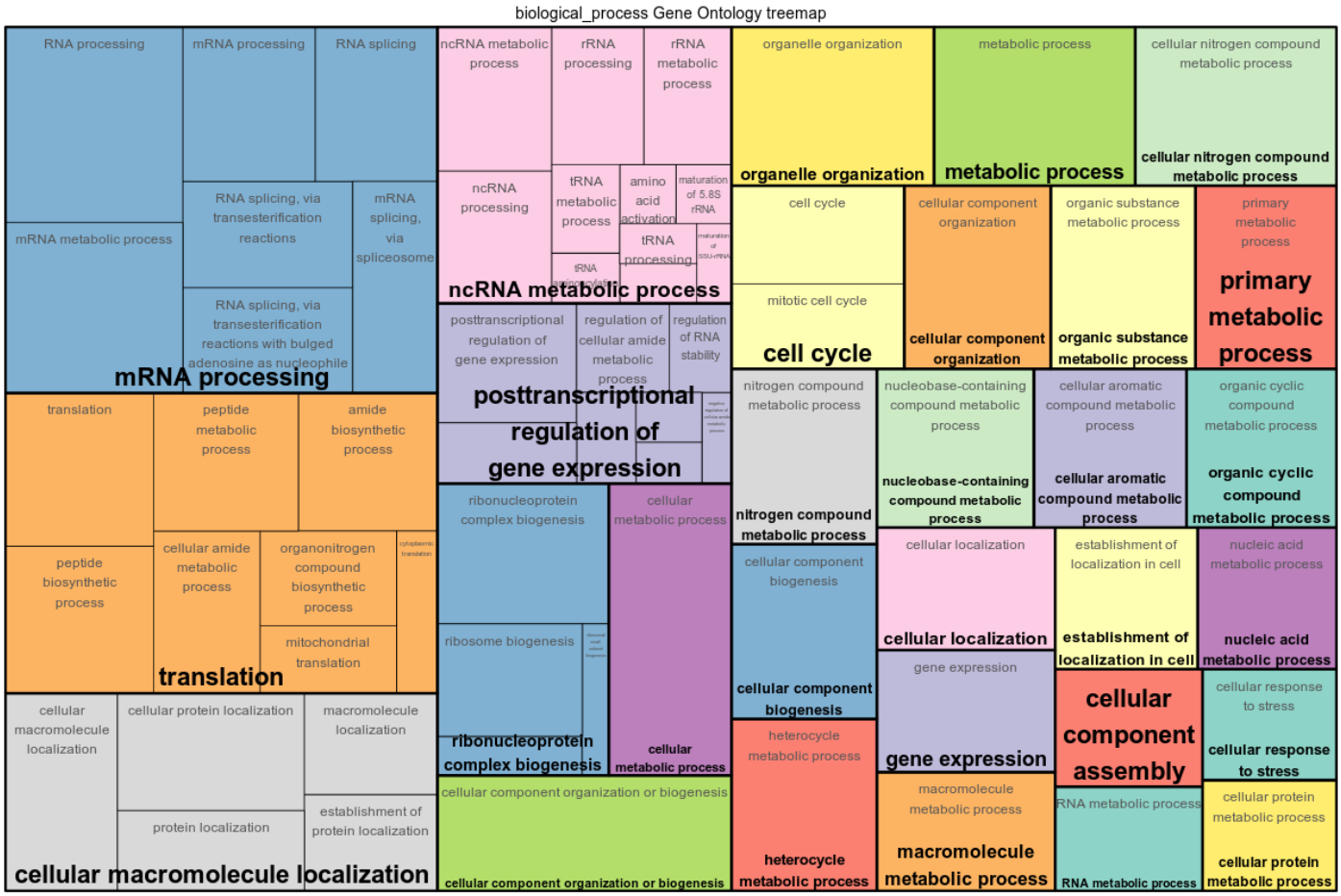


Figure 9. Enriched GO (biological process) of the list of proteins provided as input in Myc_induce.
Full classification →

TRANSPATH® Pathways (2020.2)

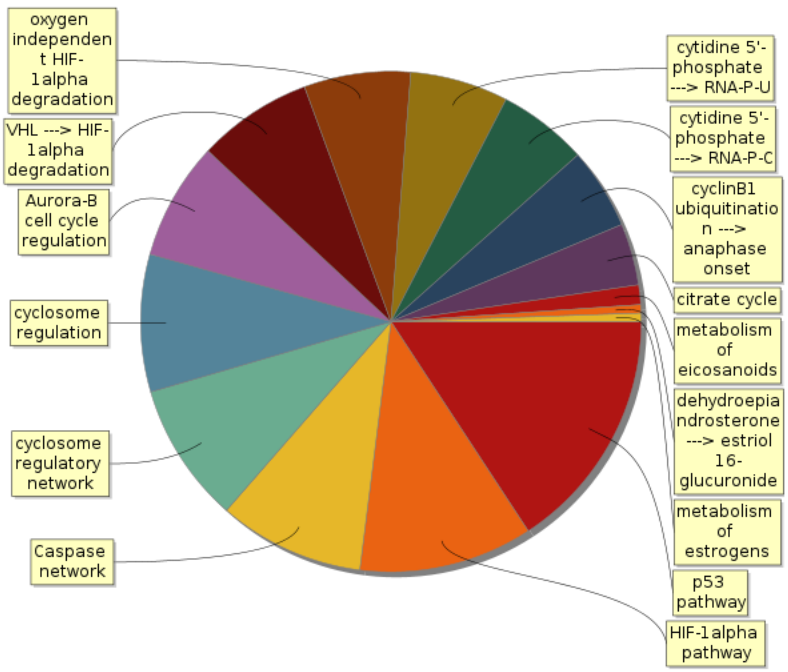


Figure 10. Enriched TRANSPATH® Pathways (2020.2) of the list of proteins provided as input in Myc_induce.
Full classification →

HumanPSD(TM) disease (2020.2)

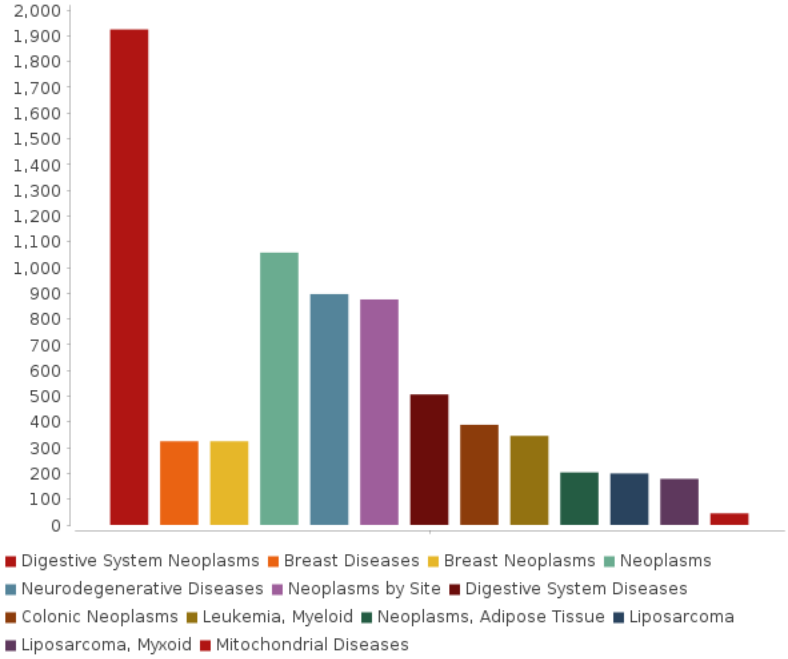


Figure 11. Enriched HumanPSD(TM) disease (2020.2) of the list of proteins provided as input in Myc_induce. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.

Full classification →

3.5. Comparison plot of transcriptome and proteome

After the analysis of transcriptome and proteome data they were compared with each other. Below we plot 9578 genes and 4660 proteins.

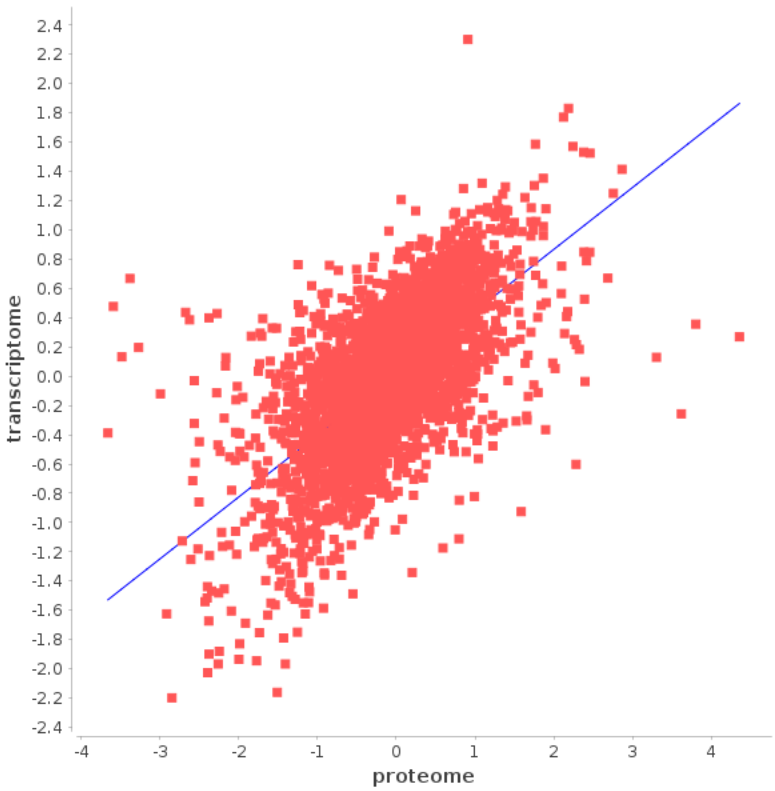


Figure 12. Comparison plot of comparison proteome vs transcriptome. X axis: protein expression value - Proteomics_avr. Y axis: LogFC of differential gene expression.

Full comparison →

Comparison of up-regulated genes (transcriptome data) and the list of proteins provided as input (proteome data)

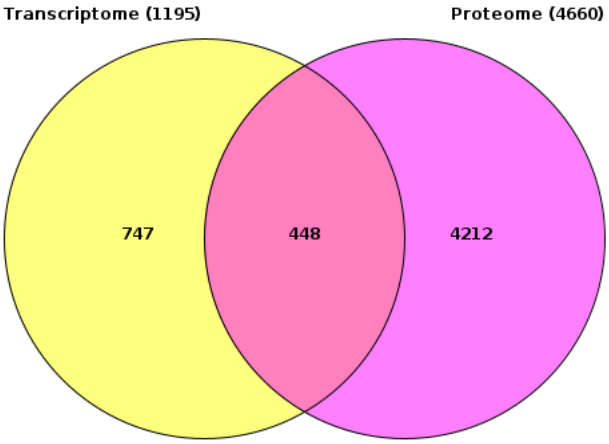


Figure 13. Intersection of up-regulated genes and the list of proteins provided as input
See full diagram →

Comparison of down-regulated genes (transcriptome data) and the list of proteins provided as input (proteome data)

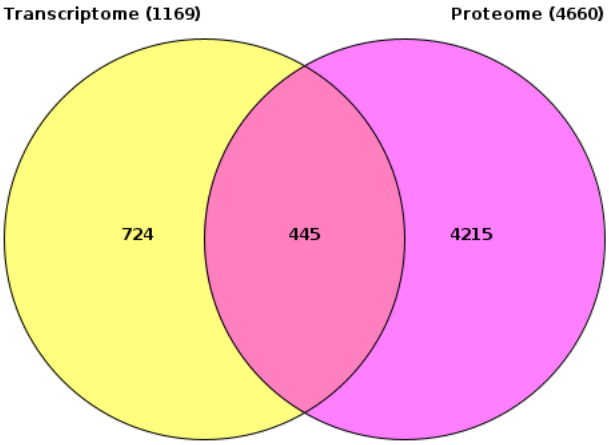
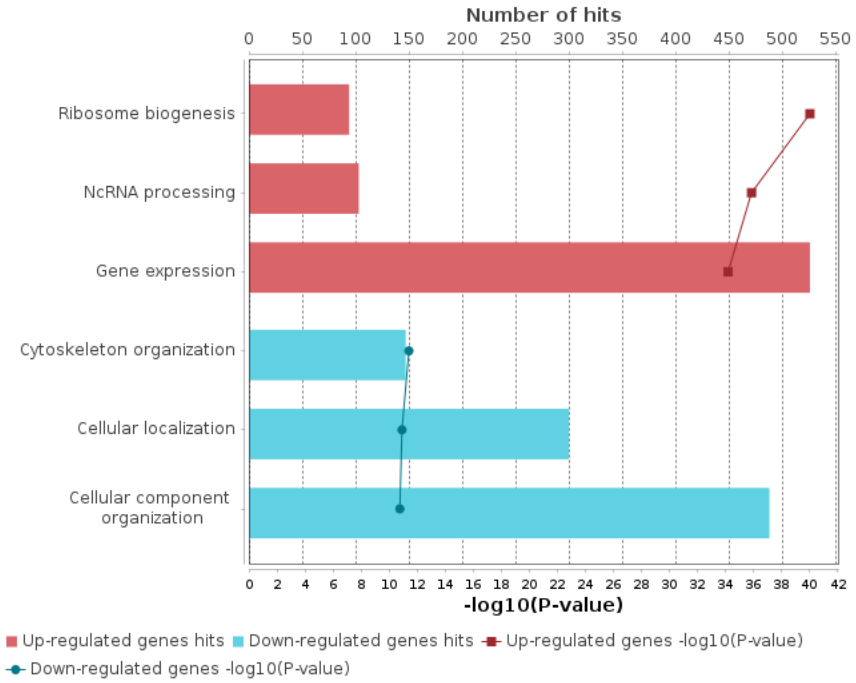


Figure 14. Intersection of down-regulated genes and the list of proteins provided as input
See full diagram →

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):



3.6. 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].

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 Myc_induce vs. Control).

To build the most specific composite modules we choose 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.

Module 1:

V$COUPTF_Q6	V$CP2_02	V$EP300_06	V$HIF2A_Q6	V$OCT4_01	V$GR_Q6	V$EGR1_09
0.00; N=3	0.00; N=3	0.00; N=2	0.00; N=2	0.00; N=2	0.00; N=2	0.87; N=3

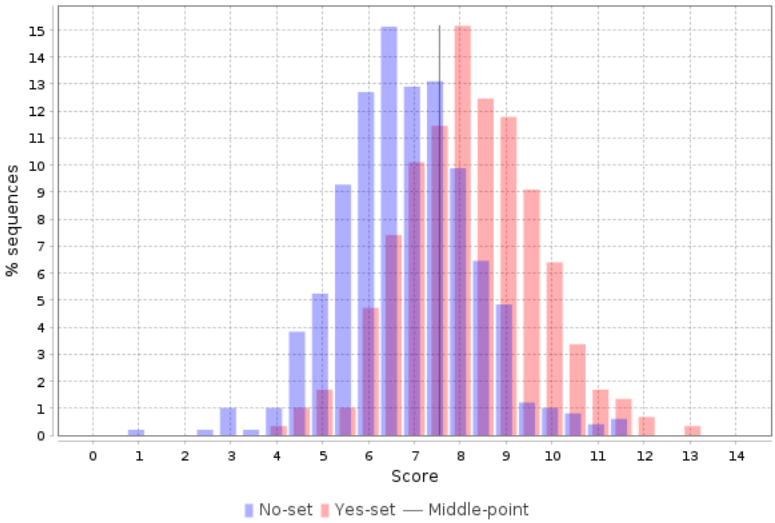
Module width: 115

Module 2:

V$SOX9_09	V$MZF1_Q5	V$IRF1_Q5	V$OCT4_01	V$RAD21_10	V$POU6F1_03
0.00; N=3	0.00; N=3	0.00; N=2	0.00; N=1	0.00; N=3	0.00; N=1

Module width: 98

Model score (-p*log10(pval)): 14.39
Wilcoxon p-value (pval): 8.65e-32
Penalty (p): 0.463
Average yes-set score: 8.17
Average no-set score: 6.84
AUC: 0.75
Middle-point: 7.55
False-positive: 28.43%
False-negative: 32.66%



See model visualization table →

Table 5. List of top ten up-regulated genes in Myc_induce vs. Control with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

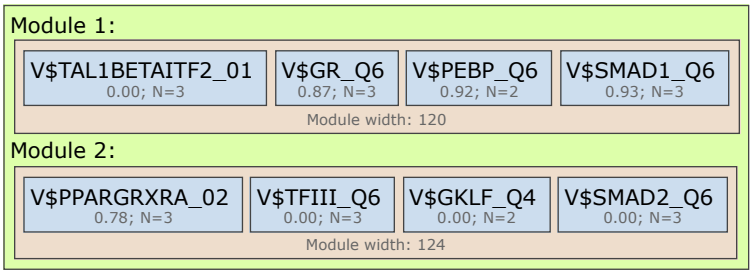
See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000110514	MADD	MAP kinase activating death domain	20.14	Sox-9(h), IRF-1(h), MZF-1(h), Rad21(h), POU6F1(h), Oct3(h), COUP-TF1(h),COUP-TF2(h)...
ENSG00000112379	ARFGEF3	ARFGEF family member 3	19.25	p300(h), Rad21(h), GR(h), Oct3(h), IRF-1(h), HIF2A(h), Sox-9(h)...
ENSG00000128564	VGF	VGF nerve growth factor inducible	19	COUP-TF1(h),COUP-TF2(h), CP2(h), GR(h), Egr-1(h), HIF2A(h), p300(h), IRF-1(h)...
ENSG00000154122	ANKH	ANKH inorganic pyrophosphate transport regulator	18.79	Egr-1(h), HIF2A(h), CP2(h), MZF-1(h), COUP-TF1(h),COUP-TF2(h), Sox-9(h), GR(h)...
ENSG00000134313	KIDINS220	kinase D interacting substrate 220	18.63	COUP-TF1(h),COUP-TF2(h), CP2(h), MZF-1(h), HIF2A(h), Rad21(h), GR(h), Sox-9(h)...
ENSG00000158050	DUSP2	dual specificity phosphatase 2	18.62	p300(h), COUP-TF1(h),COUP-TF2(h), CP2(h), Egr-1(h), HIF2A(h), GR(h), IRF-1(h)...
ENSG00000188735	TMEM120B	transmembrane protein 120B	18.39	IRF-1(h), Rad21(h), MZF-1(h), Sox-9(h), POU6F1(h), GR(h), Oct3(h)...
ENSG00000187555	USP7	ubiquitin specific peptidase 7	18.27	COUP-TF1(h),COUP-TF2(h), CP2(h), Rad21(h), HIF2A(h), GR(h), p300(h), IRF-1(h)...
ENSG00000158062	UBXN11	UBX domain protein 11	18.1	MZF-1(h), Rad21(h), Sox-9(h), Oct3(h), GR(h), p300(h), CP2(h)...
ENSG00000026297	RNASET2	ribonuclease T2	18.09	Sox-9(h), Egr-1(h), MZF-1(h), CP2(h), COUP-TF1(h),COUP-TF2(h), Rad21(h), HIF2A(h)...

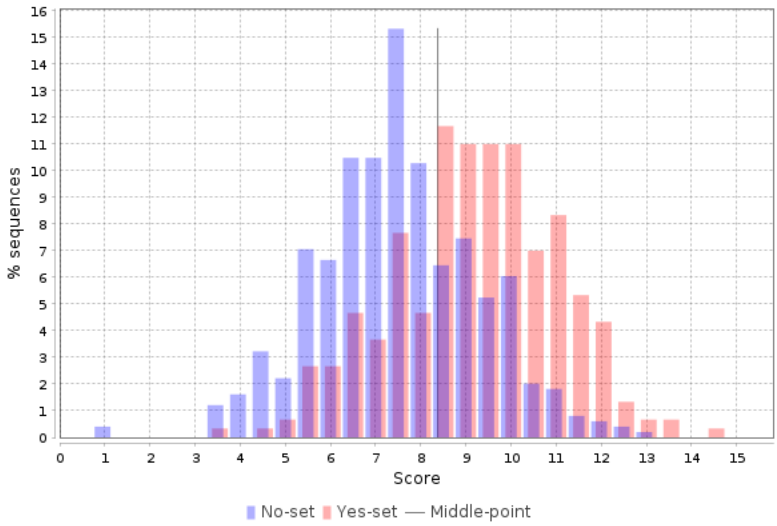
Enhancer model potentially involved in regulation of target genes (down-regulated genes in Myc_induce vs. Control).

To build the most specific composite modules we choose 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.



Model score (-p*log10(pval)): 16.74
Wilcoxon p-value (pval): 5.74e-32
Penalty (p): 0.536
Average yes-set score: 9.19
Average no-set score: 7.51
AUC: 0.75
Middle-point: 8.38
False-positive: 29.03%
False-negative: 29.00%



See model visualization table →

Table 6. List of top ten down-regulated genes in Myc_induce vs. Control 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
ENSG00000147804	SLC39A4	solute carrier family 39 member 4	15.3	PPARgamma(h),RXR-alpha(h), GR(h), ITF-2(h),Tal-1(h), Smad1(h), TFII-I(h), Smad2(h), GKLF(h)
ENSG00000113140	SPARC	secreted protein acidic and cysteine rich	14.71	Smad2(h), PPARgamma(h),RXR-alpha(h), TFII-I(h), GKLF(h), Smad1(h), GR(h), ITF-2(h),Tal-1(h)
ENSG00000068745	IP6K2	inositol hexakisphosphate kinase 2	14.7	PPARgamma(h),RXR-alpha(h), Smad2(h), GKLF(h), TFII-I(h), ITF-2(h),Tal-1(h), Smad1(h), GR(h)...
ENSG00000186193	SAPCD2	suppressor APC domain containing 2	14.62	Smad2(h), ITF-2(h),Tal-1(h), GR(h), Smad1(h), PPARgamma(h),RXR-alpha(h), TFII-I(h), GKLF(h)
ENSG00000128394	APOBEC3F	apolipoprotein B mRNA editing enzyme catalytic subunit 3F	14.58	TFII-I(h), GR(h), ITF-2(h),Tal-1(h), PPARgamma(h),RXR-alpha(h), GKLF(h), Smad1(h), Smad2(h)
ENSG00000135404	CD63	CD63 molecule	14.52	TFII-I(h), Smad2(h), GKLF(h), PPARgamma(h),RXR-alpha(h), GR(h), ITF-2(h),Tal-1(h), Smad1(h)...
ENSG00000135414	GDF11	growth differentiation factor 11	14.48	ITF-2(h),Tal-1(h), Smad1(h), PPARgamma(h),RXR-alpha(h), GR(h), Smad2(h), GKLF(h), TFII-I(h)
ENSG00000125868	DSTN	destrin, actin depolymerizing factor	14.41	TFII-I(h), PPARgamma(h),RXR-alpha(h), GKLF(h), GR(h), Smad1(h), ITF-2(h),Tal-1(h)
ENSG00000069399	BCL3	BCL3 transcription coactivator	14.12	Smad1(h), GR(h), ITF-2(h),Tal-1(h), TFII-I(h), Smad2(h), GKLF(h), PPARgamma(h),RXR-alpha(h)
ENSG00000211584	SLC48A1	solute carrier family 48 member 1	14.08	ITF-2(h),Tal-1(h), GR(h), Smad1(h), Smad2(h), PPARgamma(h),RXR-alpha(h), TFII-I(h), GKLF(h)

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 13 and 13 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 7-8).

Table 7. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in Myc_induce vs. Control). ***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 →

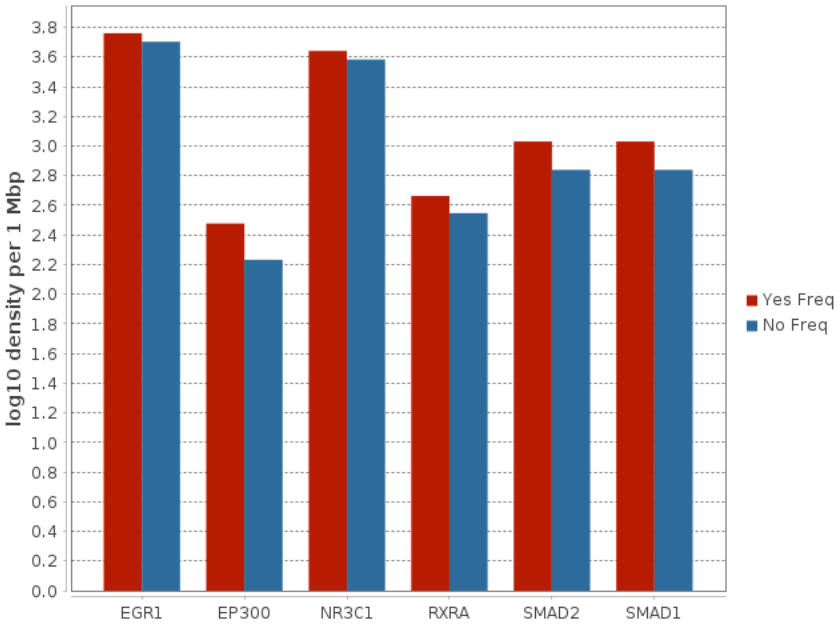
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000017914	EGR1	early growth response 1	6.43	1.14
MO000056654	EP300	E1A binding protein p300	5.1	1.76
MO000031266	NR3C1	nuclear receptor subfamily 3 group C member 1	5.05	1.14
MO000026694	EPAS1	endothelial PAS domain protein 1	4.9	1.28
MO000042938	RAD21	RAD21 cohesin complex component	4.55	1.51
MO000056618	POU5F1	POU class 5 homeobox 1	4.47	1.7
MO000018993	SOX9	SRY-box transcription factor 9	4.32	2.08
MO000117988	TFCP2	transcription factor CP2	4.25	1.38
MO000007686	IRF1	interferon regulatory factor 1	3.88	1.2
MO000024736	NR2F1	nuclear receptor subfamily 2 group F member 1	3.88	8.33

Table 8. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in Myc_induce vs. Control). ***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
MO000019619	RXRA	retinoid X receptor alpha	6.47	1.31
MO000057829	SMAD2	SMAD family member 2	6.45	1.55
MO000019609	SMAD1	SMAD family member 1	6.36	1.55
MO000033565	PPARG	peroxisome proliferator activated receptor gamma	6.24	1.32
MO000032489	TAL1	TAL bHLH transcription factor 1, erythroid differentiation factor	6.1	4.95
MO000019622	GTF2I	general transcription factor IIi	6.04	1.32
MO000031266	NR3C1	nuclear receptor subfamily 3 group C member 1	5.95	1.47
MO000025375	RUNX1	RUNX family transcription factor 1	5.88	1.88
MO000125561	KLF4	Kruppel like factor 4	4.75	1.54
MO000026238	RUNX3	RUNX family transcription factor 3	4.67	1.88

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: EGR1, EP300, NR3C1, RXRA, SMAD2 and SMAD1.



3.7. Finding master regulators in networks

In the second step of the upstream analysis common regulators of the revealed TFs were identified. Using proteomics data we selected differentially expressed proteins that are involved in signal transduction pathways and used these proteins as "context set" [4] in the algorithm of identification of master regulators. 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 9-10.

*Table 9. Master regulators that may govern the regulation of **up-regulated** genes in Myc_induce vs. Control. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and proteomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	Contained in proteome set	Total rank	logFC (transcriptome)
MO0000023365	SIRT1(h)	SIRT1	sirtuin 1	1	125	0.75
MO0000084718	SIRT1-isoform1(h)	SIRT1	sirtuin 1	1	135	0.75
MO0000032712	MKP-4(h)	DUSP9	dual specificity phosphatase 9	1	155	0.45
MO0000018901	CKII-alpha(h):CKII-alpha2(h):(CKII-beta(h))2	CSNK2A1, CSNK2A2, CSNK2B	casein kinase 2 alpha 1, casein kinase 2 alpha 2, casein kinase 2 beta	1	166	0.47
MO0000007919	CKII-alpha2(h)	CSNK2A2	casein kinase 2 alpha 2	1	204	0.47
MO0000021902	TFIIH-CAK(h)	CCNH, CDK7, MNAT1	MNAT1 component of CDK activating kinase, cyclin H, cyclin dependent kinase 7	1	250	0.63
MO0000031189	PKCdelta(h)	PRKCD	protein kinase C delta	1	250	0.86
MO0000157536	CKII-alpha(h):CKII-alpha2(h):CKII-beta(h)	CSNK2A1, CSNK2A2, CSNK2B	casein kinase 2 alpha 1, casein kinase 2 alpha 2, casein kinase 2 beta	1	256	0.47
MO0000039099	IL-1beta-p17:IL-1RI:IL-1RAcP:MyD88:tollip:IRAK-1{pS376}{pT387}:IRAK-4:IRAK-2	AC093012.1, IL1B, IL1R1, IL1RAP, IRAK1, IRAK2, MYD88, TOLLIP	MYD88 innate immune signal transduction adaptor, interleukin 1 beta, interleukin 1 receptor accessor...	1	269	1.33
MO0000059577	PKCdelta(h)	PRKCD	protein kinase C delta	1	283	0.86

*Table 10. Master regulators that may govern the regulation of **down-regulated** genes in Myc_induce vs. Control. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics and proteomics data.*
See full table →

ID	Master molecule name	Gene symbol	Gene description	Contained in proteome set	Total rank	logF (tra
MO000279336	Rac1:GTP:pak2	CYBA, CYBB, NCF1, NCF2, NCF4, PAK2, RAC1, SYTL1	Rac family small GTPase 1, cytochrome b-245 alpha chain, cytochrome b-245 beta chain, neutrophil cyt...	1	316	-1.02
MO000188147	PAK1{pS199}{pS204}{pT423}:Rac1:GTP	CYBA, CYBB, NCF1, NCF2, NCF4, PAK1, RAC1, SYTL1	Rac family small GTPase 1, cytochrome b-245 alpha chain, cytochrome b-245 beta chain, neutrophil cyt...	1	317	-1.02
MO000016807	Ras:GTP:Raf{p}	ARAF, BRAF, CYBA, CYBB, HRAS, KRAS, NCF1, NCF2, NCF4, NRAS, RAC1, RAF1, SYTL1	A-Raf proto-oncogene, serine/threonine kinase, B-Raf proto-oncogene, serine/threonine kinase, HRas p...	1	339	-1.02
MO000021274	caveolin-1(h)	CAV1	caveolin 1	1	356	-2.03
MO000161481	Cytochrome b-558:p22phox:p40phox{p}:p67phox:Rac1:GTP:JFC1:PtdIns(3,4)P2:PA:p47phox	CYBA, CYBB, NCF1, NCF2, NCF4, RAC1, SYTL1	Rac family small GTPase 1, cytochrome b-245 alpha chain, cytochrome b-245 beta chain, neutrophil cyt...	1	364	-1.02
MO000038590	Rac1:GTP:MEKK4	CYBA, CYBB, MAP3K4, NCF1, NCF2, NCF4, RAC1, SYTL1	Rac family small GTPase 1, cytochrome b-245 alpha chain, cytochrome b-245 beta chain, mitogen-activa...	1	417	-1.02
MO000129074	SHPS1(h)	SIRPA	signal regulatory protein alpha	1	433	-1.05
MO000034393	PRK1(h)	PKN1	protein kinase N1	1	449	-0.47
MO000017291	integrins	ITGA1, ITGA2B, ITGA3, ITGA4, ITGA5, ITGA6, ITGA8, ITGA9, ITGAL, ITGAV, ITGB1, ITGB2, ITGB3, ITGB4, I...	integrin subunit alpha 1, integrin subunit alpha 2b, integrin subunit alpha 3, integrin subunit alph...	1	492	-1.41
MO000022370	Pin1(h)	PIN1	peptidylprolyl cis/trans isomerase, NIMA-interacting 1	1	493	-0.77

The intracellular regulatory pathways controlled by the above-mentioned master regulators are depicted in Figures 15 and 16. 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.

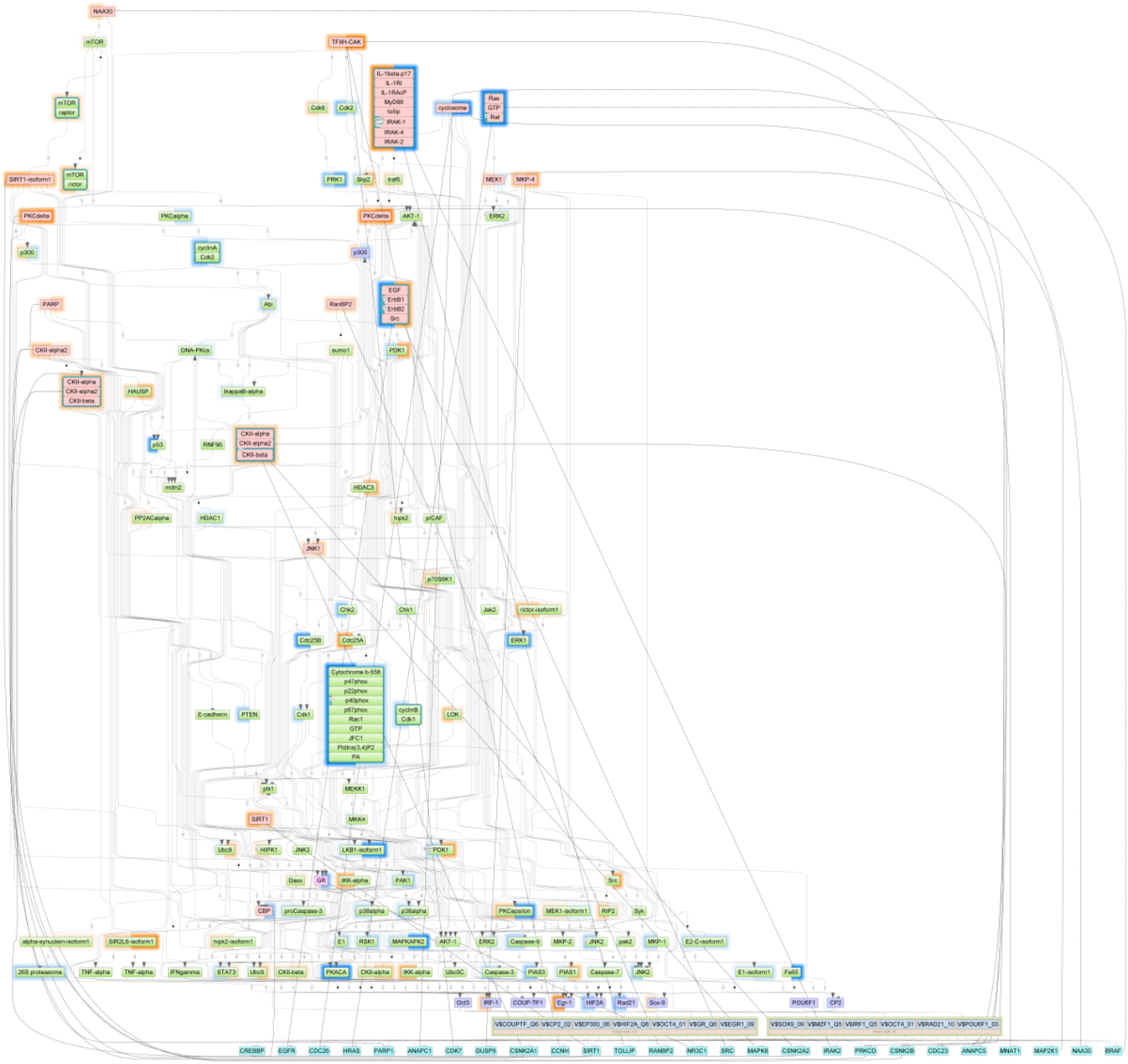


Figure 15. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in Myc_induce vs. Control. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp. The left half of a highlighting frame corresponds to transcriptomic data, the right one to proteomic data.

See full diagram →

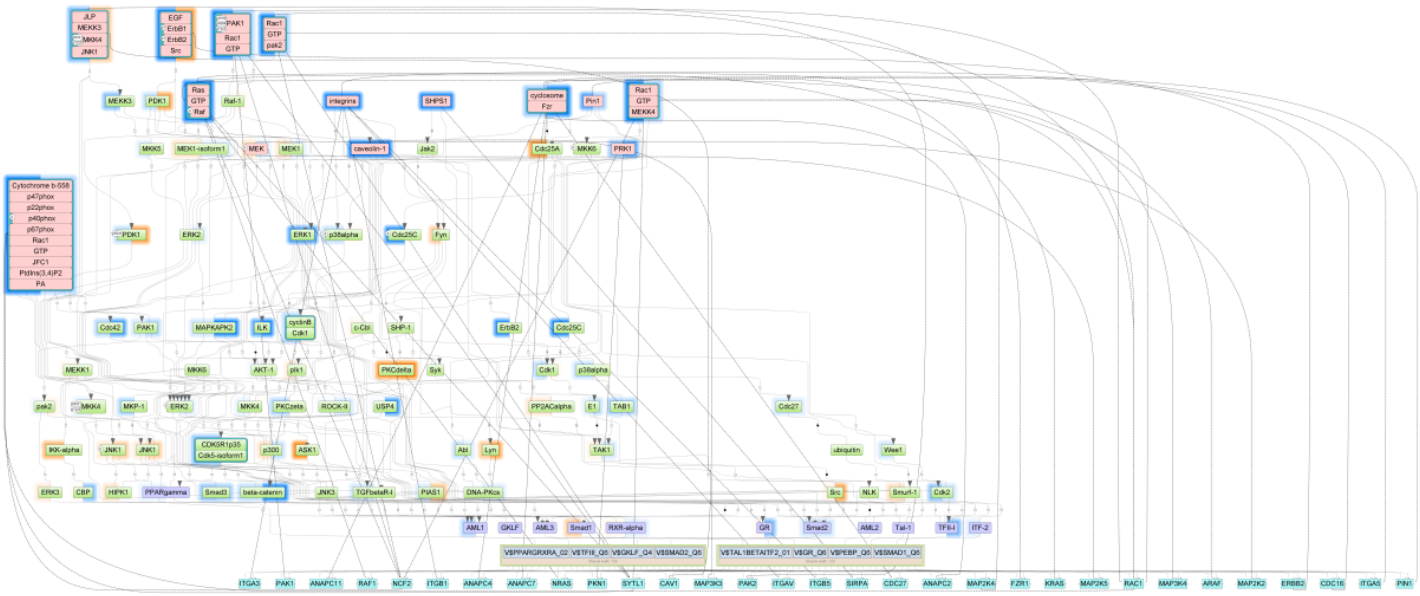


Figure 16. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in Myc_induce vs. Control. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network during the search for master regulators from selected TFs. Orange and blue frames highlight molecules that are encoded by up- and downregulated genes, resp. The left half of a highlighting frame corresponds to transcriptomic data, the right one to proteomic data.

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 11. 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	Contained in proteome set	Total rank	logFC (transcriptome)
CCND1	cyclin D1	1	1	359	0.68
ITGA4	integrin subunit alpha 4	8	1	407	0.51
YES1	YES proto-oncogene 1, Src family tyrosine kinase	1	1	428	0.51
CSNK2A2	casein kinase 2 alpha 2	1	1	450	0.47
LYN	LYN proto-oncogene, Src family tyrosine kinase	4	1	494	0.46
MERTK	MER proto-oncogene, tyrosine kinase	1	0	495	0.95

*Table 12. 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.*

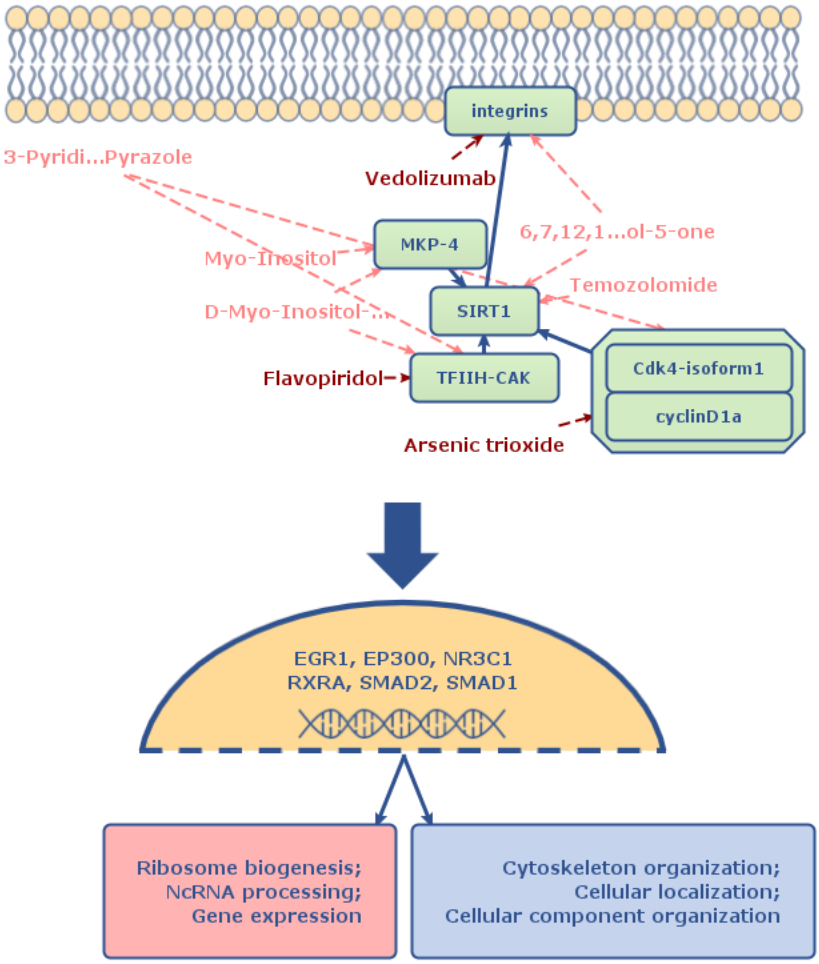
See full table →

Gene symbol	Gene Description	Druggability score	Contained in proteome set	Total rank	logFC (transcriptome)
DUSP9	dual specificity phosphatase 9	162.41	1	155	0.45
MNAT1	MNAT1 component of CDK activating kinase	382.78	1	250	0.63
CCNH	cyclin H	21.41	1	250	0.63
CCND3	cyclin D3	26.53	1	349	0.32
CCND1	cyclin D1	31.12	1	359	0.68
RPS6KB1	ribosomal protein S6 kinase B1	59.08	1	380	0.36

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:

- integrins
- SIRT1
- Cdk4-isoform1:cyclinD1a
- TFIIH-CAK
- MKP-4

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: Myo-Inositol, Flavopiridol, 3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,2-.C.]Pyrazole, D-Myo-Inositol-Hexasulphate, Arsenic trioxide, 6,7,12,13-tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazol-5-one, Vedolizumab and Temozolomide, 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 13. 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)
Bosutinib	SRC, MAP2K1, LYN	19	2	Leukemia, Myeloid	small molecule,approved
Temsirolimus	MTOR	46	5	Carcinoma, Renal Cell, Hodgkin Disease, Lymphoma, Lymphoma, Non-Hodgkin, Noma	small molecule,approved
Everolimus	MTOR	46	5	Breast Neoplasms, Carcinoma, Hepatocellular, Carcinoma, Renal Cell, Communicable Diseases, Coronary Artery Disease, Cysts, Cytomegalovirus Infections...	small molecule,approved
Prednisolone	NR3C1	84	3	Adrenal Insufficiency, Affect, Alopecia, Apnea, Arteritis, Arthritis, Arthritis, Juvenile...	small molecule,approved
Aflibercept	VEGFA	121	6	Central Serous Chorioretinopathy, Choroidal Neovascularization, Cysts, Diabetic Retinopathy, Edema, Macular Degeneration, Macular Edema...	biotech,approved

Repurposing drugs

Table 14. 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)
Ingenol Mebutate	PRKCD, PRKCA	29	Keratosis, Keratosis, Actinic	small molecule,approved
Vitamin E	PPP2CB, PRKCA, PPP2CA	33	Angina Pectoris, Variant, Asphyxia, Cicatrix, Cicatrix, Hypertrophic, Diabetes Mellitus, Dyslipidemias, Epilepsy...	small molecule,approved,nutraceutical
Dasatinib	SRC, YES1, FYN	34	Leukemia, Leukemia, Lymphoid, Leukemia, Myelogenous, Chronic, BCR-ABL Positive, Leukemia, Myeloid, Precursor Cell Lymphoblastic Leukemia-Lymphoma	small molecule,approved,investigational
Vedolizumab	ITGA4	36	Colitis, Colitis, Ulcerative, Crohn Disease, Ulcer	biotech,approved
Arsenic trioxide	IKBKB, CCND1	45	Leukemia, Leukemia, Myeloid, Leukemia, Promyelocytic, Acute	small molecule,approved,investigational

Table 15. Prospective drugs, predicted by PASS software to be active against the identified drug targets with 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
D-Myo-Inositol-Hexasulphate	PTPRR, PRPF19, MNAT1, HTRA2, TRAF6, TOPORS, TXN...	232	0.76
1-Anilino-8-Naphthalene SulfonatePTPRR, HTRA2, TXN, UBASH3B, SGK1, MAP2K3, CSNK2B...	440	0.6	
Sorafenib	RPS6KA3, MAP2K7, CSNK1A1, GRK2, PRKAA2, MAPK6, CSNK1E...	767	0.3
Risedronate	PTPRR, GRB2, BDNF, PRKAA2, MAPK6, UBASH3B, EGFR...	768	0.3
Temozolomide	SETD7, MAP2K7, CSNK1A1, PRKAA2, PRPF19, MUL1, MNAT1...	770	0.3

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
3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,...	HTRA2, TRAF6, TXN, IRAK2, TRIM27, CSNK2B, FBXW7...	29	1.7
Vatalanib	STK10, MARK3, MAP2K7, SLK, IRAK2, PARP1, SGK1...	52	1.34
6,7,12,13-tetrahydro-5H-indolo[2,3-a...	STK10, MARK3, MAP2K7, HTRA2, SLK, TXN, IRAK2...	56	2.18
N-[4-Methyl-3-[[4-(3-Pyridinyl)-2-Py...	TRAF6, IRAK2, TRIM27, CSNK2B, FBXW7, MARCH5, E4F1...	65	1.88
1,3-DIPHENYLUREA	HTRA2, TRAF6, TXN, UBE2N, IRAK2, TRIM27, CSNK2B...	83	1.11

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Bosutinib, Ingenol Mebutate, D-Myo-Inositol-Hexasulphate and 3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,2-.C.]Pyrazole. These drugs were selected for acting on the following targets: LYN, PRKCA, DUSP9 and MNAT1, 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 proteomics* data. The study is done in the context of *Neoplasm Metastasis and Osteosarcoma*. 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:

Bosutinib, Ingenol Mebutate, D-Myo-Inositol-Hexasulphate and 3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,2-.C.]Pyrazole

These drugs were selected for acting on the following targets: LYN, PRKCA, DUSP9 and MNAT1, 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:

integrins, SIRT1, Cdk4-isoform1:cyclinD1a, TFIIH-CAK and MKP-4

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: Myo-Inositol, Flavopiridol, 3-Pyridin-4-Yl-2,4-Dihydro-Indeno[1,2-.C.]Pyrazole, D-Myo-Inositol-Hexasulphate, Arsenic trioxide, 6,7,12,13-tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazol-5-one, Vedolizumab and Temozolomide. 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.

- integrins
- SIRT1
- Cdk4-isoform1:cyclinD1a
- TFIIH-CAK
- MKP-4

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 2020.2 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2020.2 (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 2020.2 (https://genexplain.com/humanpsd).
The Ensembl database release Human99.38 (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.

Genomic data processing

When analyzing a list of genomic variations (from vcf file or computed by Genome Enhancer from fastq files), first of all, we compute a specific mutation weight (w) for each variation depending on it's location in gene body and gene flanking regions (-1000 upstream and +1000 downstream of the gene body).

w = 0.7 for variations in exon area
w = 1.3 for variations in promoter region (-1000bp upstream and 100bp downstream of TSS),
w = 1.0 for variations in other locations.

Total Gene mutation weight is the sum of the weights w of all variations located inside the gene body and in the gene flanking regions.
Next, a weighted score is calculated for all genes with the following formula:
Weighted score = In_disease * In_transpath * Gene mutation weight, where

In_disease = 1.5 for genes assigned to selected diseases,
In_transpath = 2.0 for genes mapped to Transpath pathways,
and In_disease = In_transpath = 1.0 in all other cases.

At the next step, 300 genes with highest weighted score are selected for further CMA model search.
The mutation weights (w) are also used to find the regulatory regions of the genes most affected by the variations. A sliding window of 1100 bp is used to scan through the intronic, 5' and 3' regions of the genes and a region is selected with the highest sum of the mutation weights.

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.

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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 Myc_induce vs. Control).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in Myc_induce vs. Control).

Disclaimer

Decisions regarding care and treatment of patients should be fully made by attending doctors. The predicted chemical compounds listed in the report are given only for doctor's consideration and they cannot be treated as prescribed medication. It is the physician's responsibility to independently decide whether any, none or all of the predicted compounds can be used solely or in combination for patient treatment purposes, taking into account all applicable information regarding FDA prescribing recommendations for any therapeutic and the patient's condition, including, but not limited to, the patient's and family's medical history, physical examinations, information from various diagnostic tests, and patient preferences in accordance with the current standard of care. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly.

The compounds predicted to be active against the identified drug targets in the report are not guaranteed to be active against any particular patient's condition. GeneXplain GmbH does not give any assurances or guarantees regarding the treatment information and conclusions given in the report. There is no guarantee that any third party will provide a refund for any of the treatment decisions made based on these results. None of the listed compounds was checked by Genome Enhancer for adverse side-effects or even toxic effects.

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and master regulators, constructed molecular networks, lists of chemical compounds and reconstructed model of molecular mechanisms of the studied pathology are unique in respect to the used input data set and Genome Enhancer pipeline parameters used for the current run.