PRLR and PLK4 are promising druggable targets for treating Parkinson Disease that control activity of HSF2, POU2F1 and RAD21 transcription factors on promoters of differentially expressed genes

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Genome Enhancer release 2.2 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2020.3)

Abstract

In the present study we applied the software package "Genome Enhancer" to a data set that contains *transcriptomics* data. The study is done in the context of *Parkinson Disease*. 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: HSF2, POU2F1, AR, RAD21 and ESR2. The subsequent network analysis suggested

- Caspase-8
- integrins
- nek10:Raf-1{pS338}:MEK1{pS218}{pS222}
- plk4
- prlr:tec:Vav
- Caspase-8

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: Pioglitazone, Peginterferon alfa-2a, Lipoic Acid 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
GSE145804_DESeq2_final	Transcriptomics

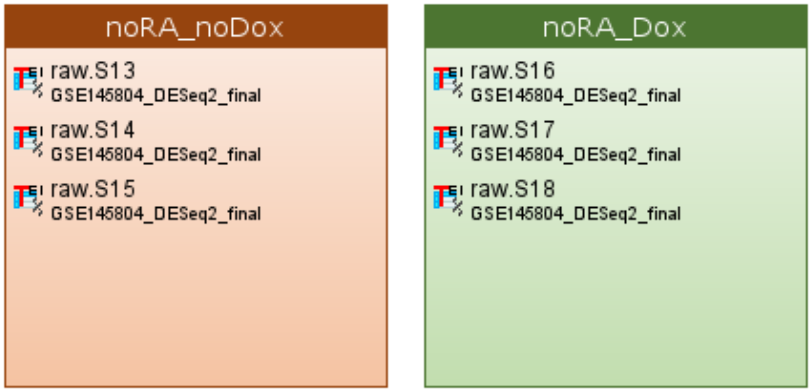


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

3. Results

We have compared the following conditions: noRA_Dox *versus* noRA_noDox.

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 edgeR tool (R/Bioconductor package integrated into our pipeline) and compared gene expression in the following sets: "noRA_Dox" with "noRA_noDox". edgeR 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 8084 upregulated genes (LogFC>0) out of which 578 genes were found as significantly upregulated (p-value<0.1) and 8862 downregulated genes (LogFC<0) out of which 726 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 noRA_Dox vs. noRA_noDox.*
See full table →

ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000145335	SNCA	synuclein alpha	4.38	10.36	2.71E-88	4.59E-84
ENSG00000118785	SPP1	secreted phosphoprotein 1	2.98	-0.28	6.23E-7	2.11E-3
ENSG00000169282	KCNAB1	potassium voltage-gated channel subfamily A member regulatory beta subunit 1	2.91	0.49	1.32E-7	5.61E-4
ENSG00000162692	VCAM1	vascular cell adhesion molecule 1	2.67	-4.05E-2	1.1E-5	2.34E-2
ENSG00000237280	AL136982.3	novel transcript	2.15	0.2	1.42E-4	0.16
ENSG00000214892	USP8P1	USP8 pseudogene 1	1.81	0.19	4.4E-3	0.83
ENSG00000224837	GCSHP5	glycine cleavage system protein H pseudogene 5	1.72	-0.1	1.84E-3	0.6
ENSG00000243300	null	null	1.65	0.44	2.8E-3	0.68
ENSG00000229474	PATL2	PAT1 homolog 2	1.53	-0.14	7.97E-3	0.98
ENSG00000236813	BTF3P8	basic transcription factor 3 pseudogene 8	1.53	-0.48	9.95E-3	0.98

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

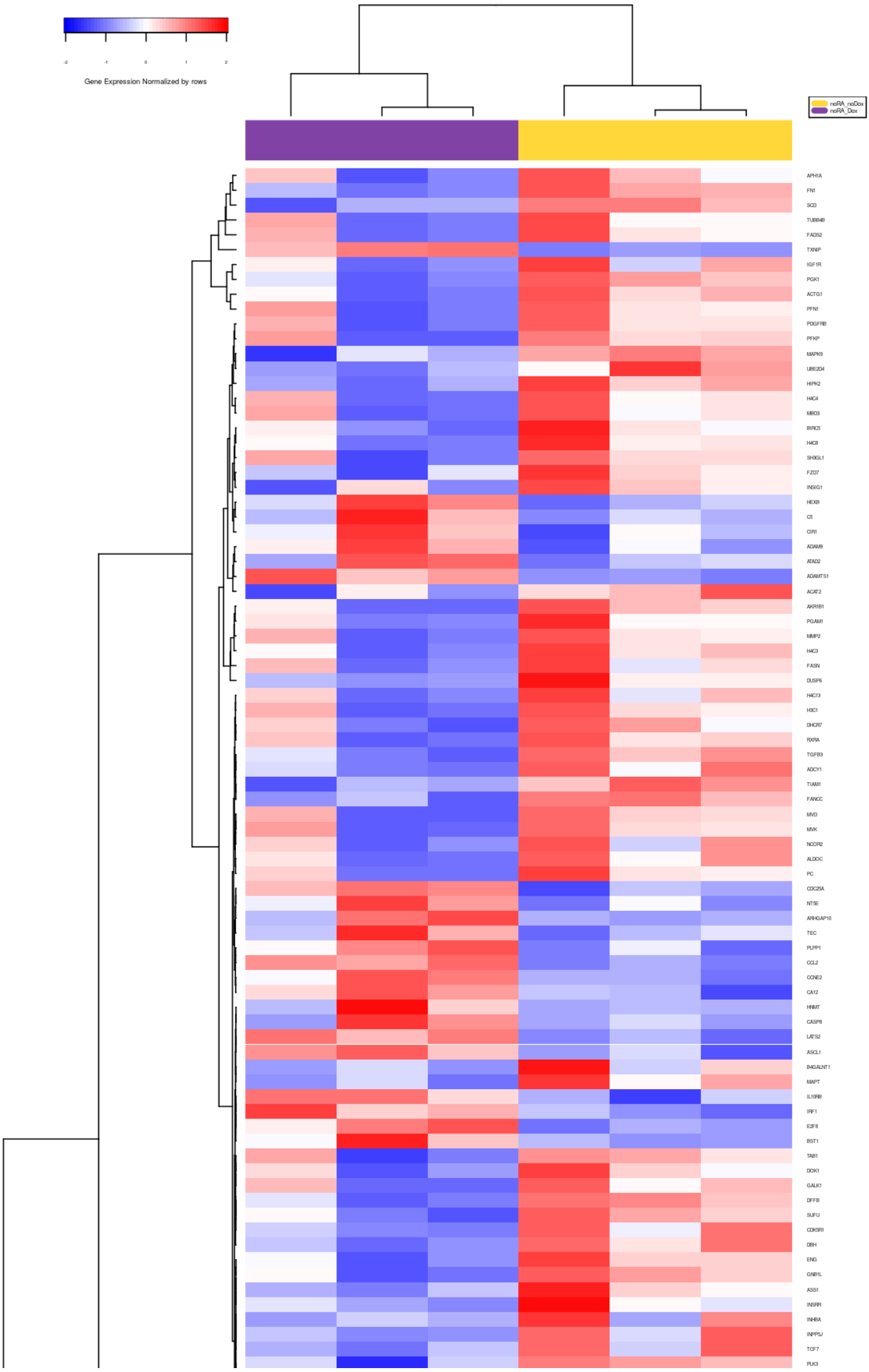
ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000186081	KRT5	keratin 5	-10.36	2.57	9.35E-7	2.64E-3
ENSG00000257594	GALNT4	polypeptide N-acetylgalactosaminyltransferase 4	-2.37	1.39E-2	4.31E-5	6.08E-2
ENSG00000167244	IGF2	insulin like growth factor 2	-2.13	-0.49	1.23E-3	0.47
ENSG00000255115	AP002812.4	family with sequence similarity 162, member A (FAM162A) pseudogene	-1.97	-0.38	2.22E-3	0.61
ENSG00000134955	SLC37A2	solute carrier family 37 member 2	-1.9	-0.42	9.67E-3	0.98
ENSG00000111679	PTPN6	protein tyrosine phosphatase non-receptor type 6	-1.9	-0.13	4.43E-3	0.83
ENSG00000179846	NKPD1	NTPase KAP family P-loop domain containing 1	-1.87	-0.33	3.5E-3	0.79
ENSG00000137801	THBS1	thrombospondin 1	-1.83	0.49	4.4E-3	0.83
ENSG00000269054	AC012313.6	novel transcript, antisense to ZNF497	-1.82	-9.51E-2	1.69E-3	0.56
ENSG00000224886	AL132656.1	novel pseudogene	-1.82	-0.35	6.76E-3	0.98

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 noRA_Dox vs. noRA_noDox

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
noRA_noDox
noRA_Dox
APH1A
FN1
SCD
TUBB4B
FADS2
TXNIP
IGF1R
PGK1
ACTG1
PFN1
PDGFRB
PFKP
MAPK9
UBE2D4
HIPK2
H4C4
MBD3
BIRC5
H4C8
SH3GL1
FZD7
INSIG1
HEXB
C5
CIR1
ADAM9
ATAD2
ADAMTS1
ACAT2
AKR1B1
PGAM1
MMP2
H4C3
FASN
DUSP6
H4C13
H3C1
DHCR7
RXRA
TGFB3
ADCY1
TIAM1
FANCC
MVD
MVK
NCOR2
ALDOC
PC
CDC25A
NT5E
ARHGAP10
TEC
PLPP1
CCL2
CCNE2
CA12
HNMT
CASP8
LATS2
ASCL1
B4GALNT1
MAPT
IL10RB
IRF1
E2F8
BST1
TAB1
DOK1
GALK1
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INSRR
INHBA
INPP5J
TCF7
PLK3

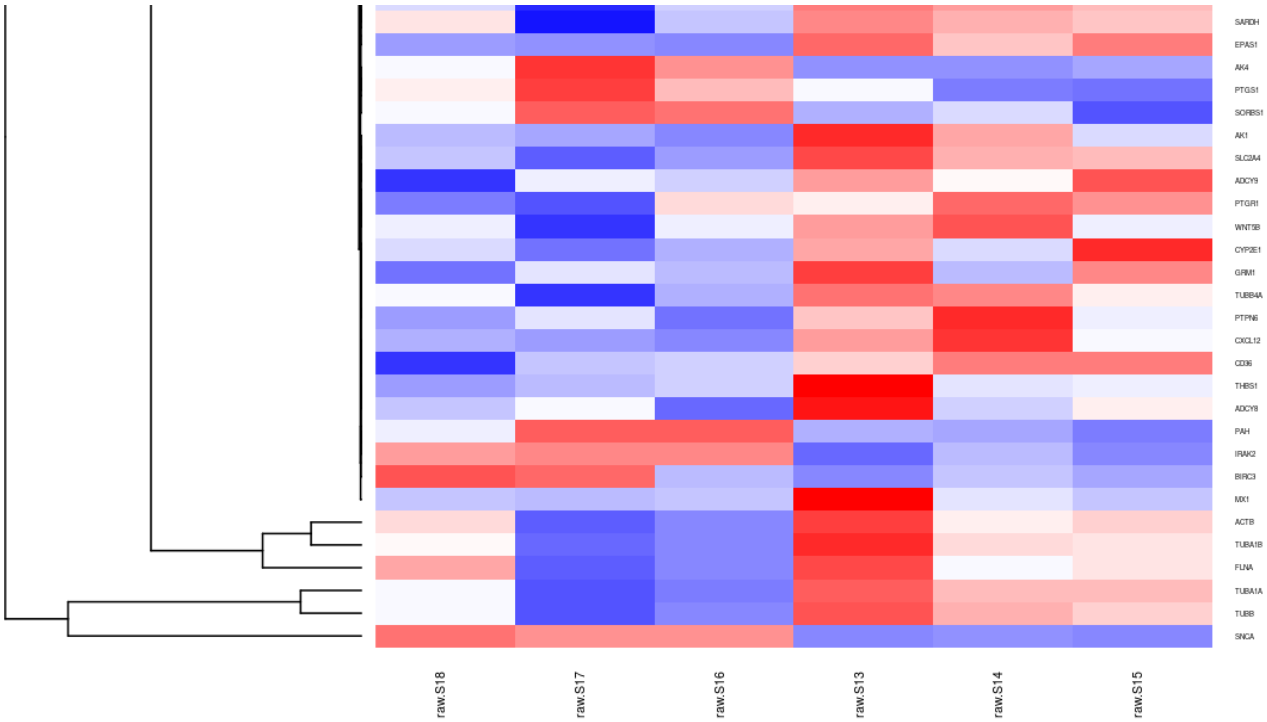


Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.

See full diagram →

Up-regulated genes in noRA_Dox vs. noRA_noDox:

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

GO (biological process)

Figure 3. Enriched GO (biological process) of up-regulated genes in noRA_Dox vs. noRA_noDox.

Full classification →

TRANSPATH® Pathways (2020.3)

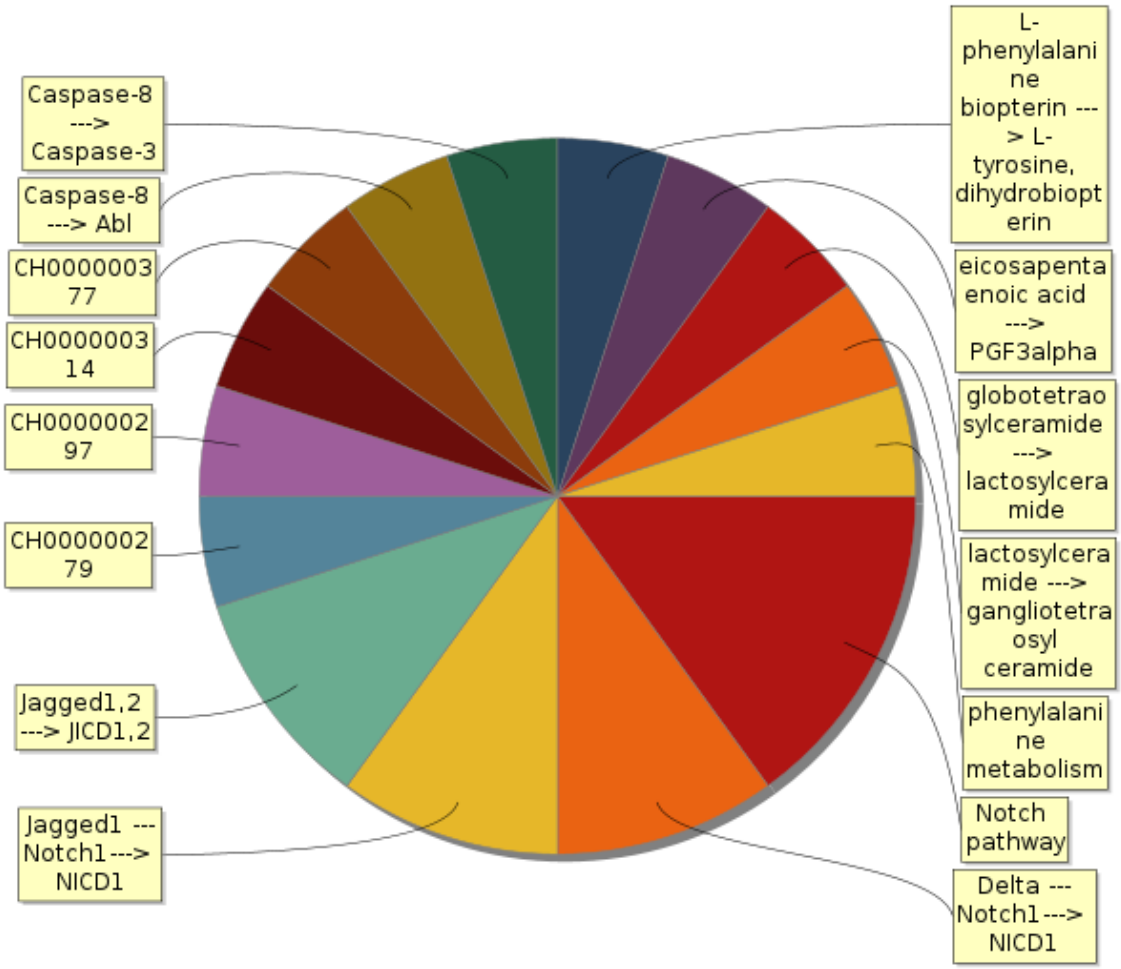


Figure 4. Enriched TRANSPATH® Pathways (2020.3) of up-regulated genes in noRA_Dox vs. noRA_noDox.
Full classification →

HumanPSD(TM) disease (2020.3)

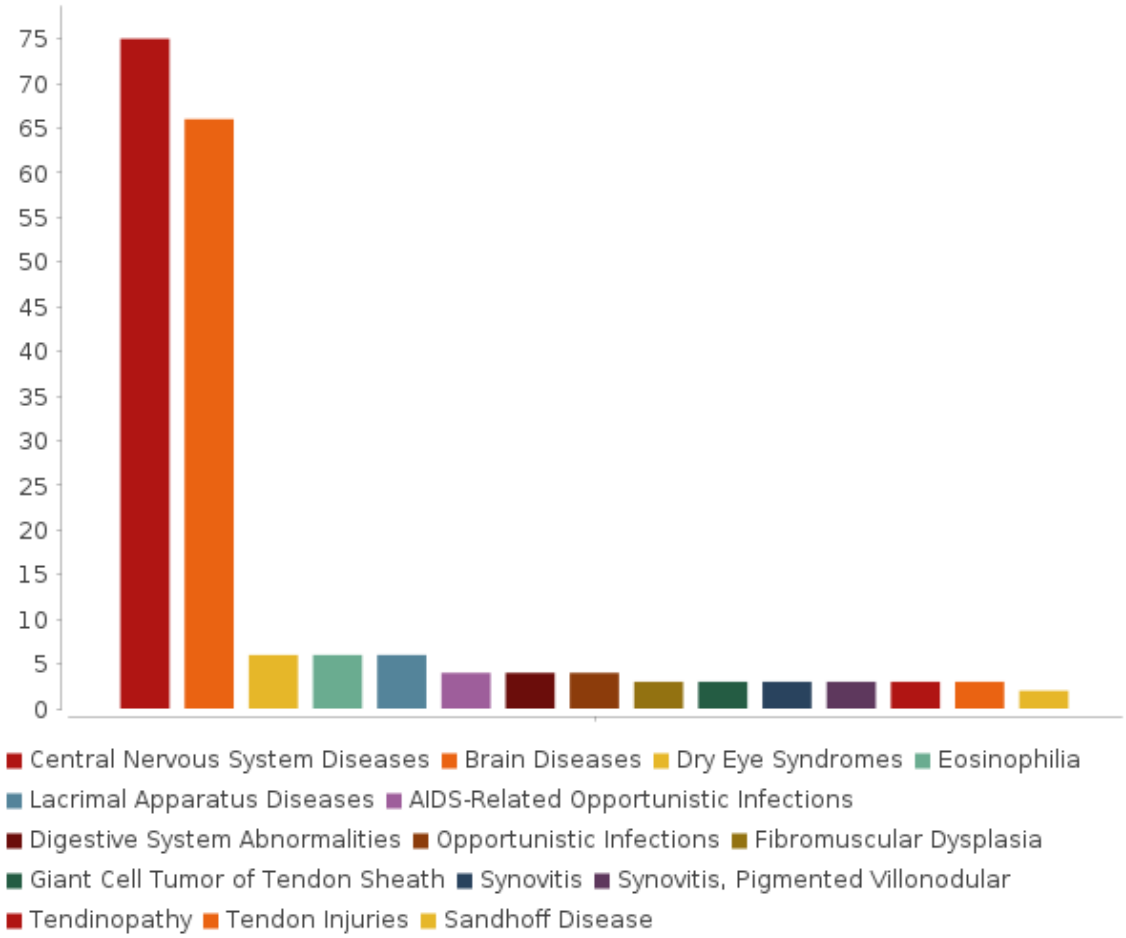


Figure 5. Enriched HumanPSD(TM) disease (2020.3) of up-regulated genes in noRA_Dox vs. noRA_noDox. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.

Full classification →

Down-regulated genes in noRA_Dox vs. noRA_noDox:

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

GO (biological process)

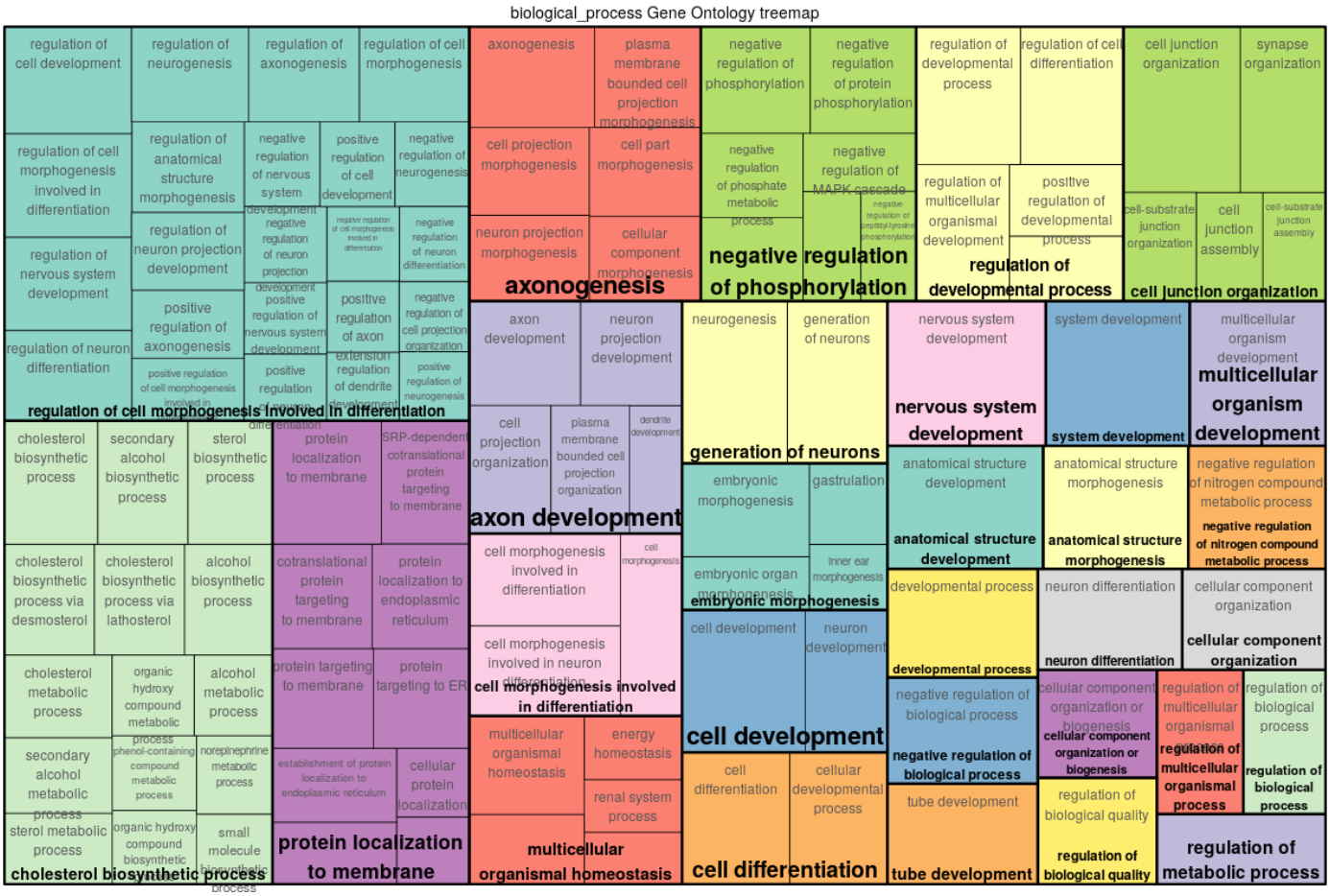


Figure 6. Enriched GO (biological process) of down-regulated genes in noRA_Dox vs. noRA_noDox.

Full classification →

TRANSPATH® Pathways (2020.3)

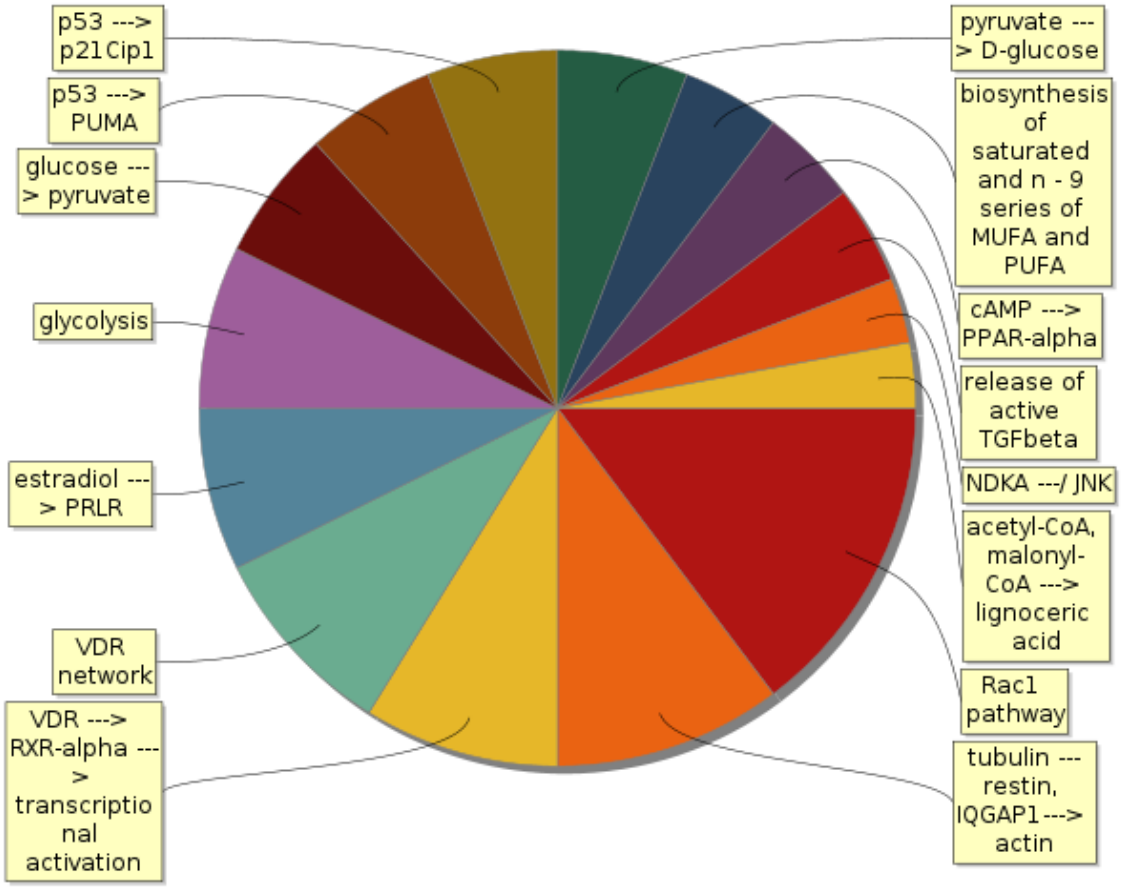


Figure 7. Enriched TRANSPATH® Pathways (2020.3) of down-regulated genes in noRA_Dox vs. noRA_noDox.
Full classification →

HumanPSD(TM) disease (2020.3)

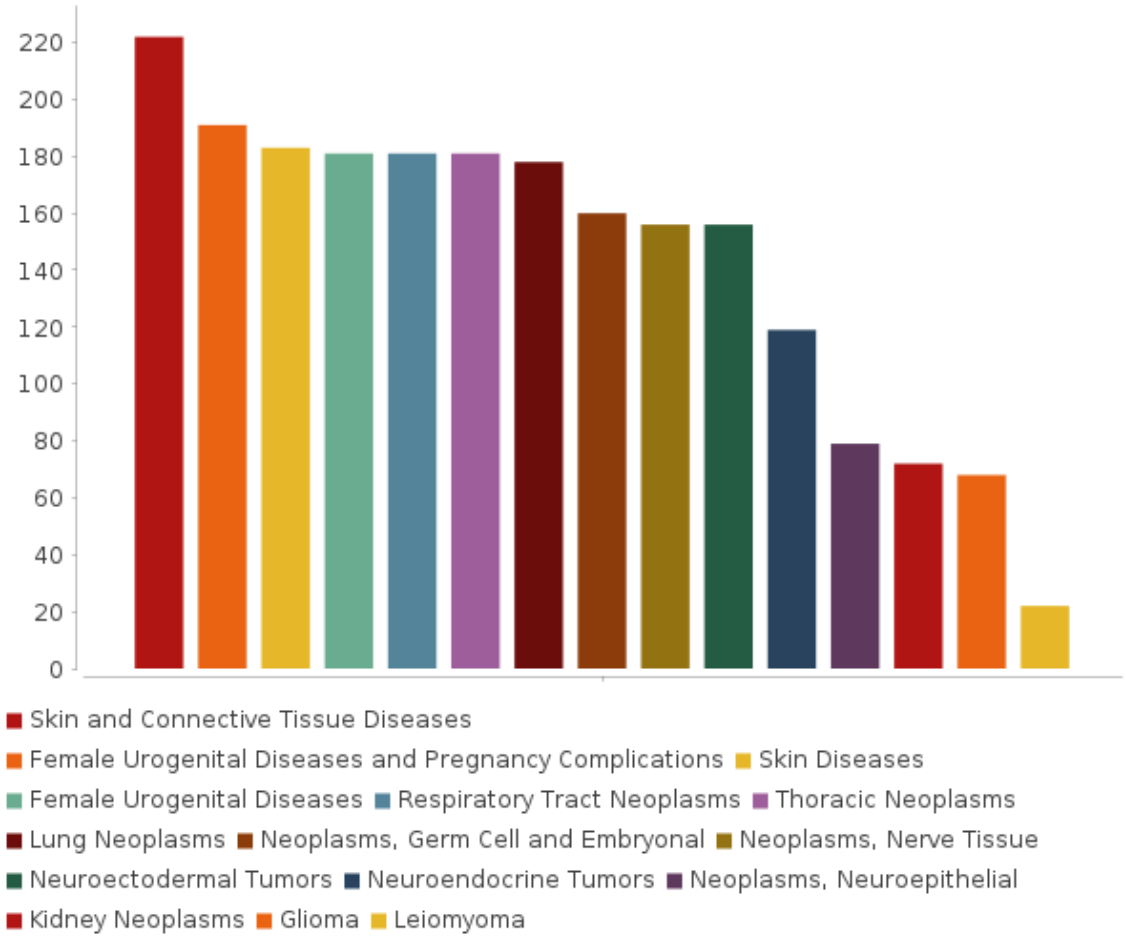
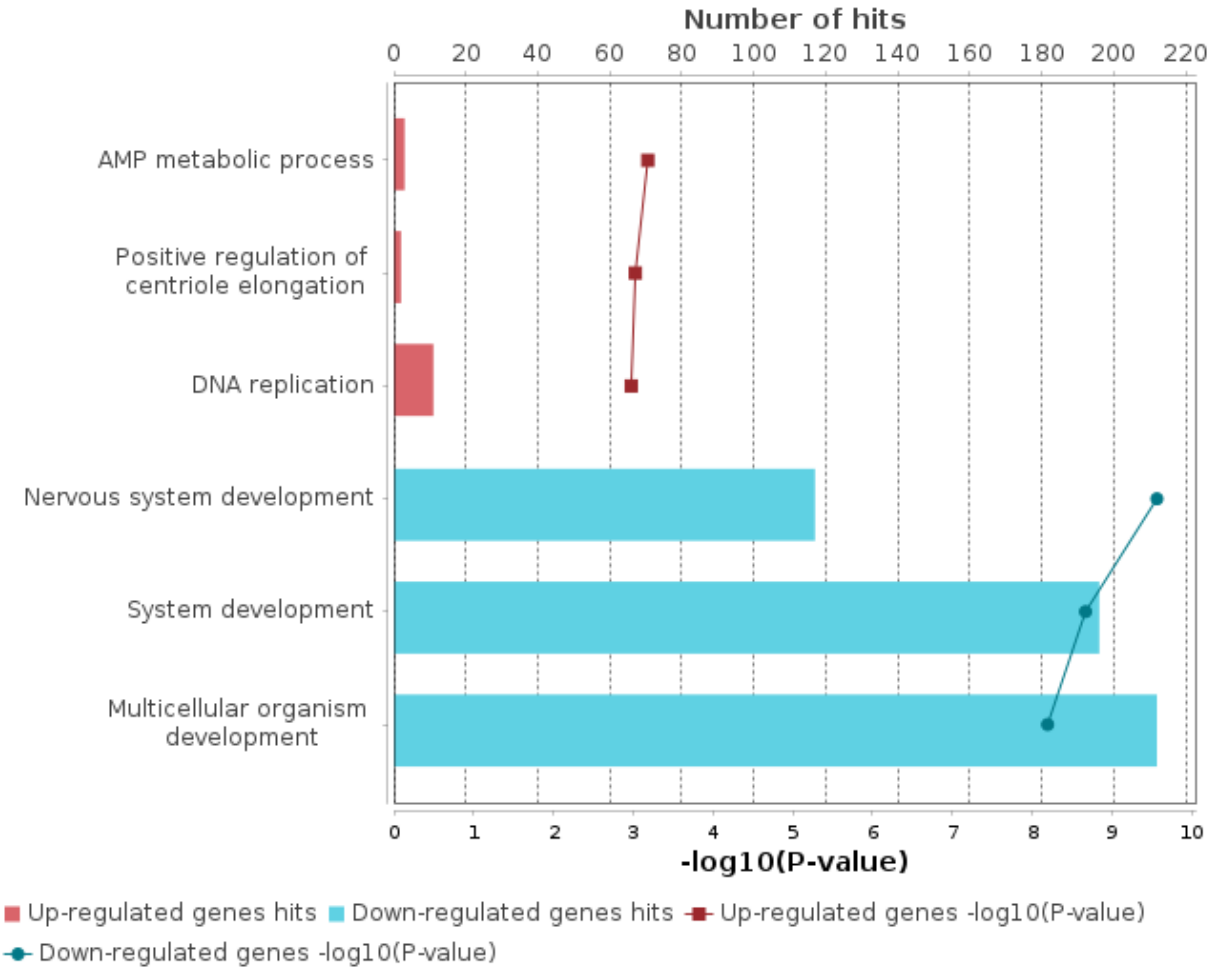


Figure 8. Enriched HumanPSD(TM) disease (2020.3) of down-regulated genes in noRA_Dox vs. noRA_noDox. The size of the bars correspond to the number of bio-markers of the given disease found among the input set.

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):



3.3. 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 noRA_Dox vs. noRA_noDox).

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.

Module 1:

V$YY1_02	V$GRE_C	V$AR_10	V$SOX9_03	V$HSF2_01	V$OCT1_Q5_01
0.77; N=3	0.80; N=1	0.91; N=1	0.69; N=2	0.97; N=1	0.91; N=1

Module width: 155

Module 2:

V$IRF1_05	V$PPARG_02	V$HNF3A_Q6	V$POU6F1_02	V$STAT1STAT1_Q3	V$TBX5_02
0.84; N=1	0.64; N=2	0.97; N=2	0.78; N=2	0.87; N=3	0.95; N=2

Module width: 136

Model score (-p*log10(pval)): 15.07
Wilcoxon p-value (pval): 1.75e-32
Penalty (p): 0.475
Average yes-set score: 5.97
Average no-set score: 4.42
AUC: 0.75
Middle-point: 5.28
False-positive: 31.40%
False-negative: 29.05%

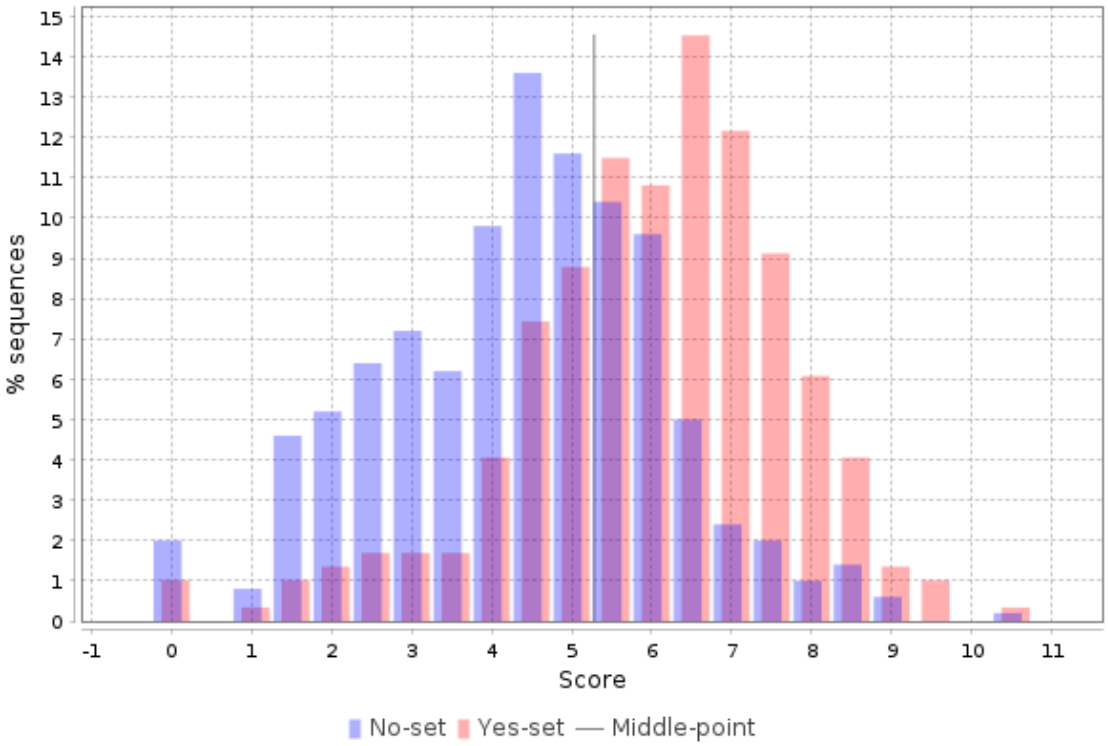


Table 4. List of top ten up-regulated genes in noRA_Dox vs. noRA_noDox with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000124380	SNRNP27	small nuclear ribonucleoprotein U4/U6.U5 subunit 27	11.55	YY1(h), Sox-9(h), GR(h), HSF2(h), POU6F1(h), PPARgamma(h), HNF-3alpha(h)...
ENSG00000183055	FAM133CP	family with sequence similarity 133 member C, pseudogene	11.1	Tbx5(h), PPARgamma(h), HNF-3alpha(h), POU6F1(h), Sox-9(h), POU2F1(h), YY1(h)...
ENSG00000139675	HNRNPA1L2	heterogeneous nuclear ribonucleoprotein A1 like 2	11.01	GR(h), AR(h), POU6F1(h), IRF-1(h), HNF-3alpha(h), PPARgamma(h), Tbx5(h)...
ENSG00000199266	SNORA60	small nucleolar RNA, H/ACA box 60	10.75	POU6F1(h), Sox-9(h), PPARgamma(h), YY1(h), IRF-1(h), HNF-3alpha(h), GR(h)...
ENSG00000224993	RPL29P12	ribosomal protein L29 pseudogene 12	10.62	Sox-9(h), YY1(h), PPARgamma(h), POU2F1(h), GR(h), AR(h), POU6F1(h)...
ENSG00000206228	HNRNPA1P4	heterogeneous nuclear ribonucleoprotein A1 pseudogene 4	10.62	YY1(h), POU6F1(h), PPARgamma(h), HNF-3alpha(h), Tbx5(h), HSF2(h), GR(h)...
ENSG00000283236	AC074141.1	novel zinc finger protein pseudogene	10.48	IRF-1(h), HNF-3alpha(h), PPARgamma(h), YY1(h), AR(h), POU6F1(h), POU2F1(h)...
ENSG00000133460	SLC2A11	solute carrier family 2 member 11	10.45	Tbx5(h), YY1(h), POU2F1(h), GR(h), AR(h), Sox-9(h), POU6F1(h)...
ENSG00000213700	RPL17P50	ribosomal protein L17 pseudogene 50	10.41	POU2F1(h), YY1(h), Tbx5(h), GR(h), AR(h), HSF2(h), HNF-3alpha(h)...
ENSG00000130844	ZNF331	zinc finger protein 331	10.3	YY1(h), HNF-3alpha(h), GR(h), AR(h), POU6F1(h), Tbx5(h), PPARgamma(h)...

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

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$RAD21_10	V$CP2_Q6	V$GLI_Q2	V$ERBETA_Q5	V$OCT1_04
0.94; N=3	0.97; N=3	0.81; N=2	0.81; N=2	0.84; N=3

Module width: 103

Module 2:

V$MAFK_Q3	V$REST_06	V$CP2_02	V$SMAD1_Q6
0.85; N=3	0.89; N=3	0.81; N=3	0.93; N=2

Module width: 114

Model score (-p*log10(pval)): 16.65
Wilcoxon p-value (pval): 6.48e-33
Penalty (p): 0.517
Average yes-set score: 8.71
Average no-set score: 7.12
AUC: 0.75
Middle-point: 8.23
False-positive: 22.60%
False-negative: 36.27%

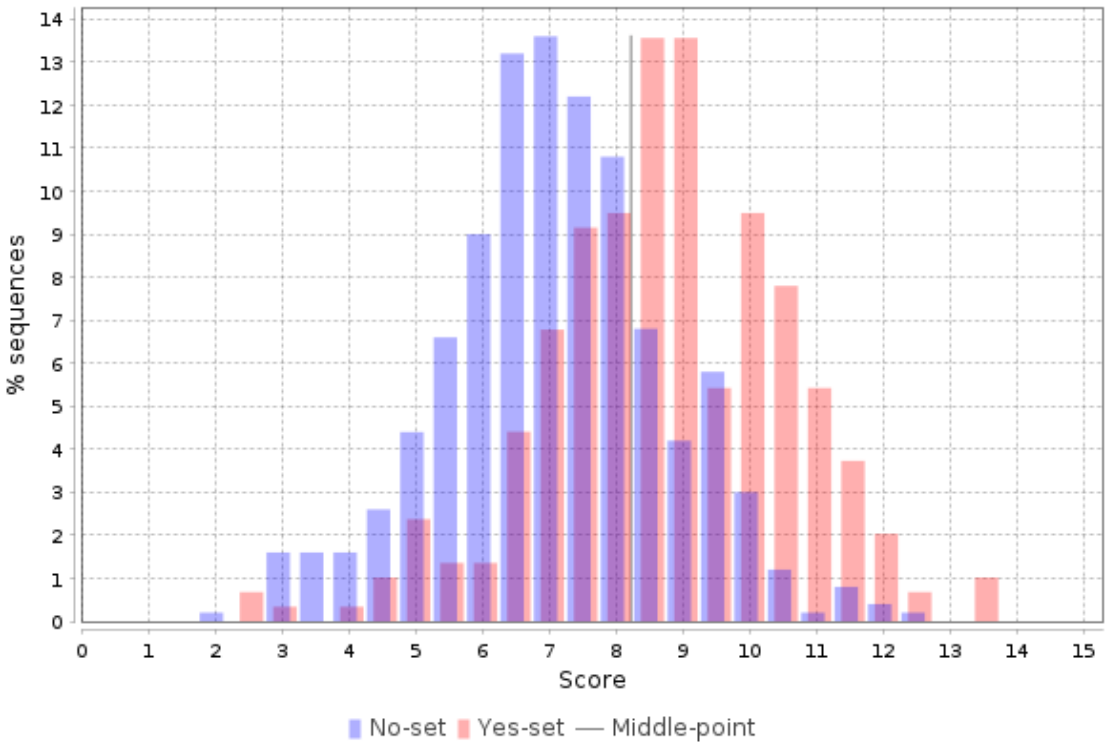


Table 5. List of top ten down-regulated genes in noRA_Dox vs. noRA_noDox with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000143793	C1orf35	chromosome 1 open reading frame 35	14.93	CP2(h), Smad1(h), REST(h), MafK(h), ER-beta(h), Rad21(h), GLI(h),GLIS1(h),Gli2(h),gli3(h)
ENSG00000111664	GNB3	G protein subunit beta 3	14.18	MafK(h), Smad1(h), CP2(h), REST(h), Rad21(h), ER-beta(h), GLI(h),GLIS1(h),Gli2(h),gli3(h)...
ENSG00000277103	AL445670.1	sorting nexin 18 (SNX18) pseudogene	13.83	MafK(h), Smad1(h), CP2(h), ER-beta(h), Rad21(h), GLI(h),GLIS1(h),Gli2(h),gli3(h), REST(h)
ENSG00000229081	LINC01165	long intergenic non-protein coding RNA 1165	13.79	ER-beta(h), CP2(h), GLI(h),GLIS1(h),Gli2(h),gli3(h), Rad21(h), Smad1(h), REST(h)
ENSG00000154358	OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	13.73	GLI(h),GLIS1(h),Gli2(h),gli3(h), CP2(h), ER-beta(h), Rad21(h), REST(h), Smad1(h)
ENSG00000121716	PILRB	paired immunoglobin like type 2 receptor beta	13.64	GLI(h),GLIS1(h),Gli2(h),gli3(h), ER-beta(h), Rad21(h), CP2(h), REST(h), Smad1(h), MafK(h)
ENSG00000103254	ANTKMT	adenine nucleotide translocase lysine methyltransferase	13.61	MafK(h), REST(h), CP2(h), GLI(h),GLIS1(h),Gli2(h),gli3(h), Rad21(h), ER-beta(h), Smad1(h)
ENSG00000267980	AC007292.1	novel transcript, antisense to SH3GL1	13.61	CP2(h), Rad21(h), ER-beta(h), GLI(h),GLIS1(h),Gli2(h),gli3(h), MafK(h), Smad1(h), REST(h)
ENSG00000281530	AC004461.3	DiGeorge syndrome critical region gene 12 (non-protein coding)	13.61	GLI(h),GLIS1(h),Gli2(h),gli3(h), POU2F1(h), CP2(h), REST(h), Rad21(h), Smad1(h), ER-beta(h)
ENSG00000258634	AL160006.1	novel transcript, antisense to ALX3	13.58	MafK(h), Smad1(h), Rad21(h), CP2(h), GLI(h),GLIS1(h),Gli2(h),gli3(h), ER-beta(h), POU2F1(h)...

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 11 and 11 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 6-7).

*Table 6. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in noRA_Dox vs. noRA_noDox). **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 →

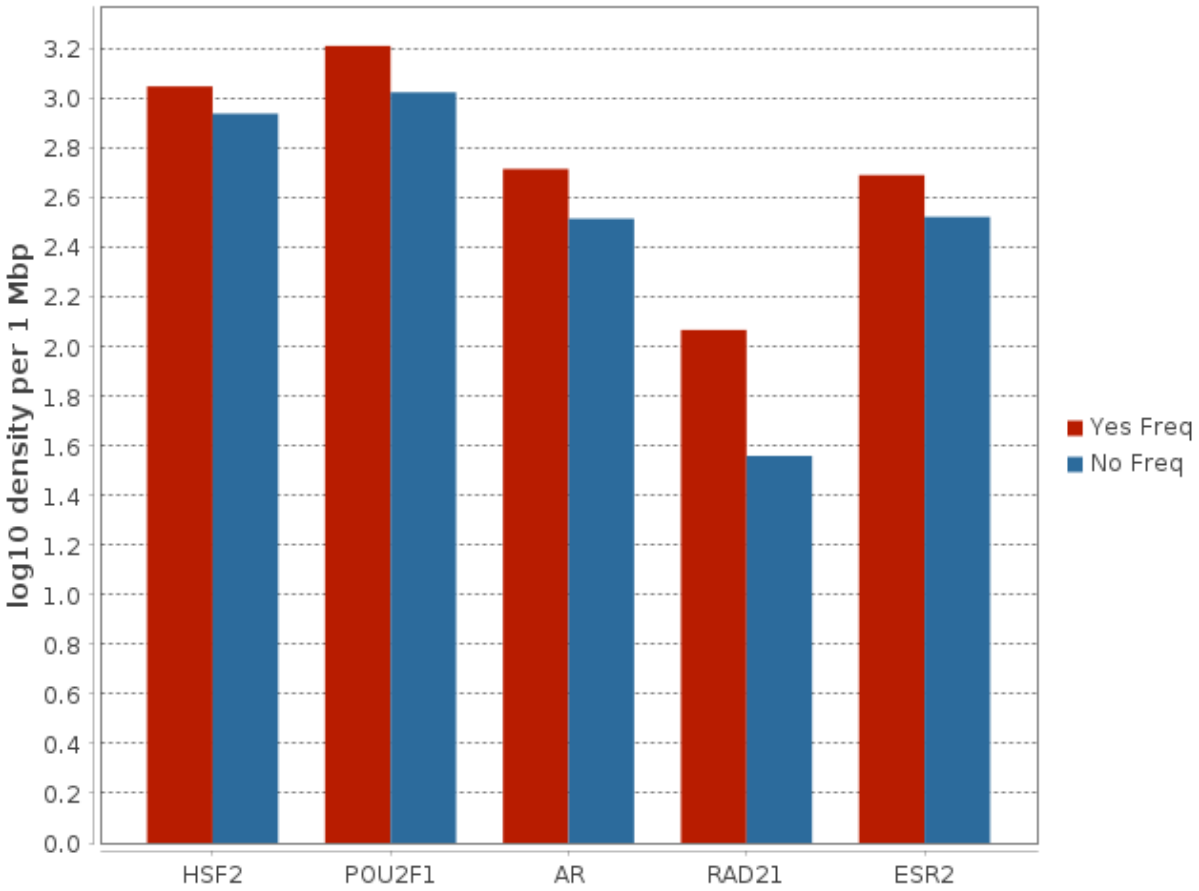
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000046011	HSF2	heat shock transcription factor 2	5.31	1.29
MO000025003	POU2F1	POU class 2 homeobox 1	4.93	1.54
MO000021454	AR	androgen receptor	4.87	1.59
MO000031266	NR3C1	nuclear receptor subfamily 3 group C member 1	4.43	1.32
MO000078913	YY1	YY1 transcription factor	4.2	1.29
MO000033565	PPARG	peroxisome proliferator activated receptor gamma	3.91	1.57
MO000018993	SOX9	SRY-box transcription factor 9	3.52	1.75
MO000007686	IRF1	interferon regulatory factor 1	3.35	1.34
MO000028320	null	null	2.83	1.46
MO000026492	FOXA1	forkhead box A1	0	1.44

*Table 7. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in noRA_Dox vs. noRA_noDox). **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
MO000042938	RAD21	RAD21 cohesin complex component	3.86	3.22
MO000025003	POU2F1	POU class 2 homeobox 1	3.71	
MO000059335	ESR2	estrogen receptor 2	3.31	1.47
MO000041817	REST	RE1 silencing transcription factor	3.16	1.54
MO000019609	SMAD1	SMAD family member 1	3.02	1.41
MO000117988	TFCP2	transcription factor CP2	2.97	1.4
MO000019117	GLI1	GLI family zinc finger 1	2.34	1.39
MO000028668	MAFK	MAF bZIP transcription factor K	2.25	1.5
MO000019113	GLI3	GLI family zinc finger 3	2.11	1.38
MO000117573	GLI2	GLI family zinc finger 2	0	1.38

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: HSF2, POU2F1, AR, RAD21 and ESR2.



3.4. 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 8-9.

*Table 8. Master regulators that may govern the regulation of **up-regulated** genes in noRA_Dox vs. noRA_noDox. **Total rank** is the sum of the ranks of the master molecules sorted by keynode score, CMA score, transcriptomics data.*

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO000020219	Caspase-8(h)	CASP8	caspase 8	0.55	87
MO000021036	Caspase-8(h)	CASP8	caspase 8	0.55	170
MO000038894	(Caspase-8)2	CASP8	caspase 8	0.55	198
MO000078269	cIAP-2(h)	BIRC3	baculoviral IAP repeat containing 3	0.62	211
MO000032073	cIAP-2(h)	BIRC3	baculoviral IAP repeat containing 3	0.62	216
MO000043221	Caspase-8a(h)	CASP8	caspase 8	0.55	228
MO000043414	cyclosome(h)	ANAPC1, ANAPC10, ANAPC11, ANAPC2, ANAPC4, ANAPC5, ANAPC7, CDC16, CDC23, CDC26, CDC27	anaphase promoting complex subunit 1, anaphase promoting complex subunit 10, anaphase promoting comp...	0.23	246
MO000021902	TFIIH-CAK(h)	CCNH, CDK7, MNAT1	MNAT1 component of CDK activating kinase, cyclin H, cyclin dependent kinase 7	0.23	276
MO000104136	cyclosome(h):Fzr1(h)	ANAPC1, ANAPC10, ANAPC11, ANAPC2, ANAPC4, ANAPC5, ANAPC7, CDC16, CDC23, CDC26, CDC27, FZR1	anaphase promoting complex subunit 1, anaphase promoting complex subunit 10, anaphase promoting comp...	0.23	278
MO000046011	HSF2(h)	HSF2	heat shock transcription factor 2	0.22	285

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

See full table →

ID	Master molecule name	Gene symbol	Gene description	logFC	Total rank
MO000017421	AKT-1(h)	AKT1	AKT serine/threonine kinase 1	-0.41	115
MO000031101	plk3(h)	PLK3	polo like kinase 3	-0.54	132
MO000034546	AKT-1(h){pT308}{pS473}	AKT1	AKT serine/threonine kinase 1	-0.41	155
MO000138699	plk3(h)	PLK3	polo like kinase 3	-0.54	155
MO000058481	AKT-1-isoform1(h)	AKT1	AKT serine/threonine kinase 1	-0.41	156
MO000033313	PKACA(h)	PRKACA	protein kinase cAMP-activated catalytic subunit alpha	-0.42	160
MO000104136	cyclosome(h):Fzr1(h)	ANAPC1, ANAPC10, ANAPC11, ANAPC2, ANAPC4, ANAPC5, ANAPC7, CDC16, CDC23, CDC26, CDC27, FZR1	anaphase promoting complex subunit 1, anaphase promoting complex subunit 10, anaphase promoting comp...	-0.54	166
MO000031189	PKCdelta(h)	PRKCD	protein kinase C delta	-0.34	175
MO000043414	cyclosome(h)	ANAPC1, ANAPC10, ANAPC11, ANAPC2, ANAPC4, ANAPC5, ANAPC7, CDC16, CDC23, CDC26, CDC27	anaphase promoting complex subunit 1, anaphase promoting complex subunit 10, anaphase promoting comp...	-0.54	182
MO000041952	calpain-1(h)	CAPN1	calpain 1	-0.48	239

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.

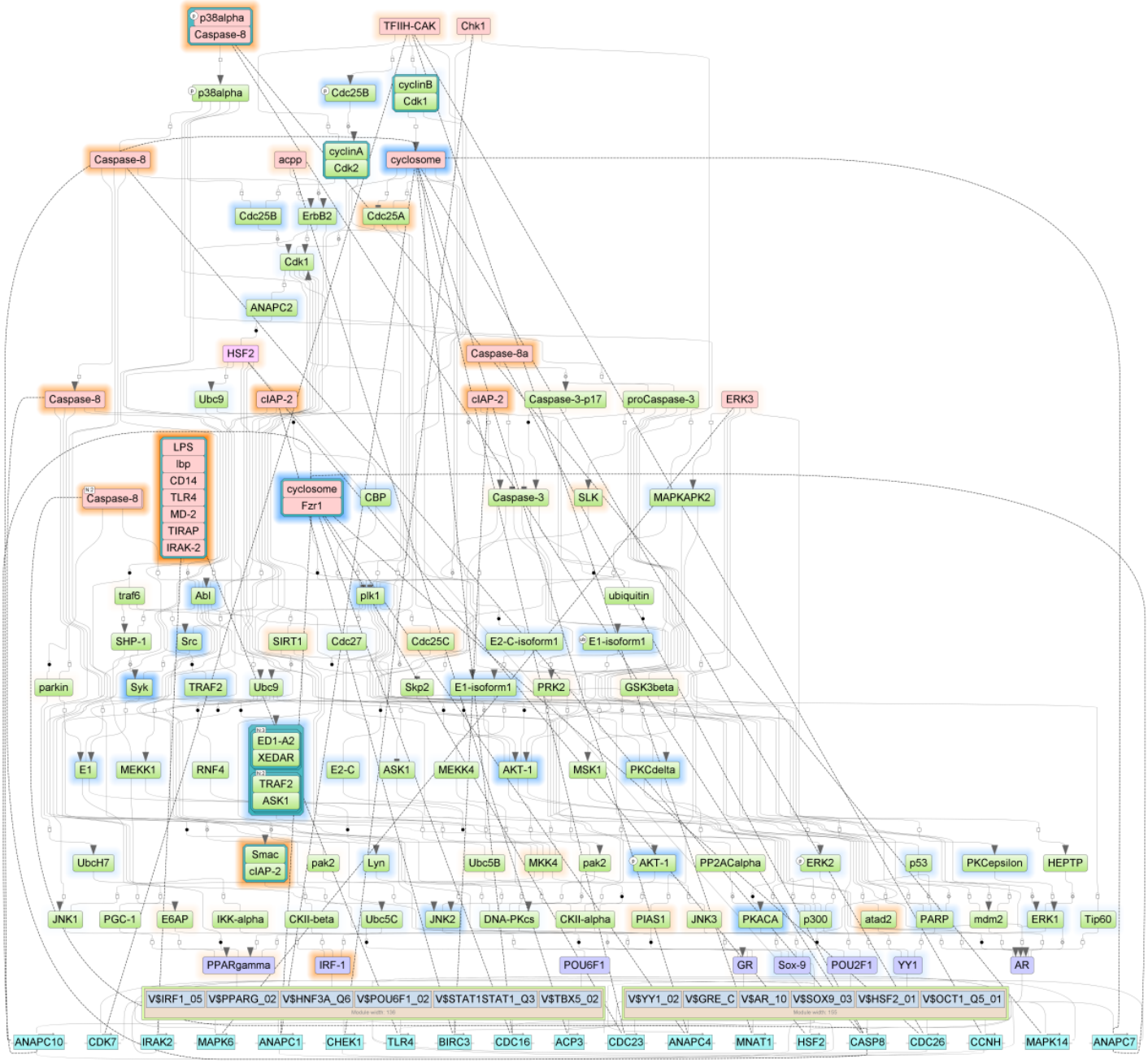


Figure 9. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in noRA_Dox vs. noRA_noDox. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network 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 →

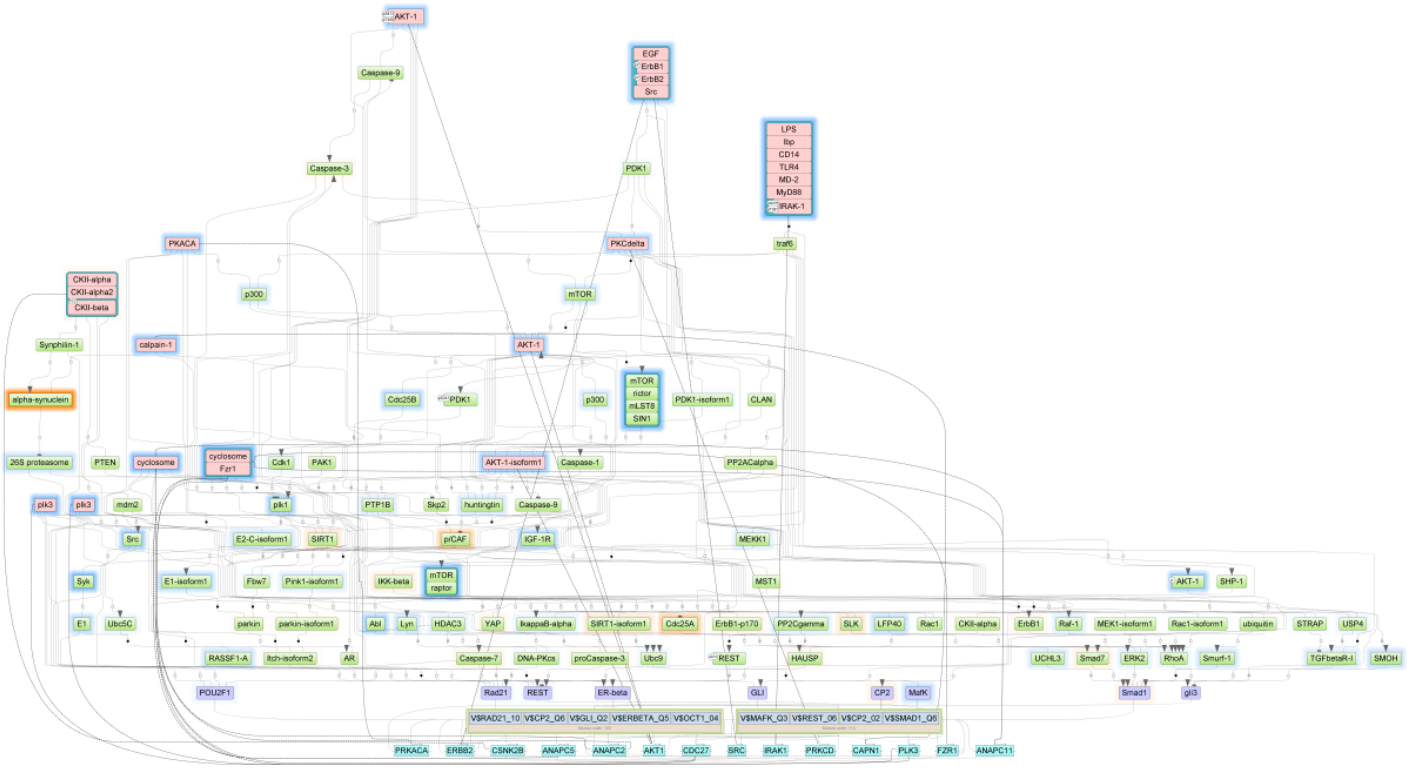


Figure 10. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in noRA_Dox vs. noRA_noDox. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules, which have been added to the network 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 10. 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
PRLR	prolactin receptor	2	0.52	486
ITGA4	integrin subunit alpha 4	8	0.22	496
PRKCQ	protein kinase C theta	3	0.32	514
CLK1	CDC like kinase 1	2	0.37	516
LCMT1	leucine carboxyl methyltransferase 1	1	0.11	540
ROCK1	Rho associated coiled-coil containing protein kinase 1	4	0.2	573

*Table 11. 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.*

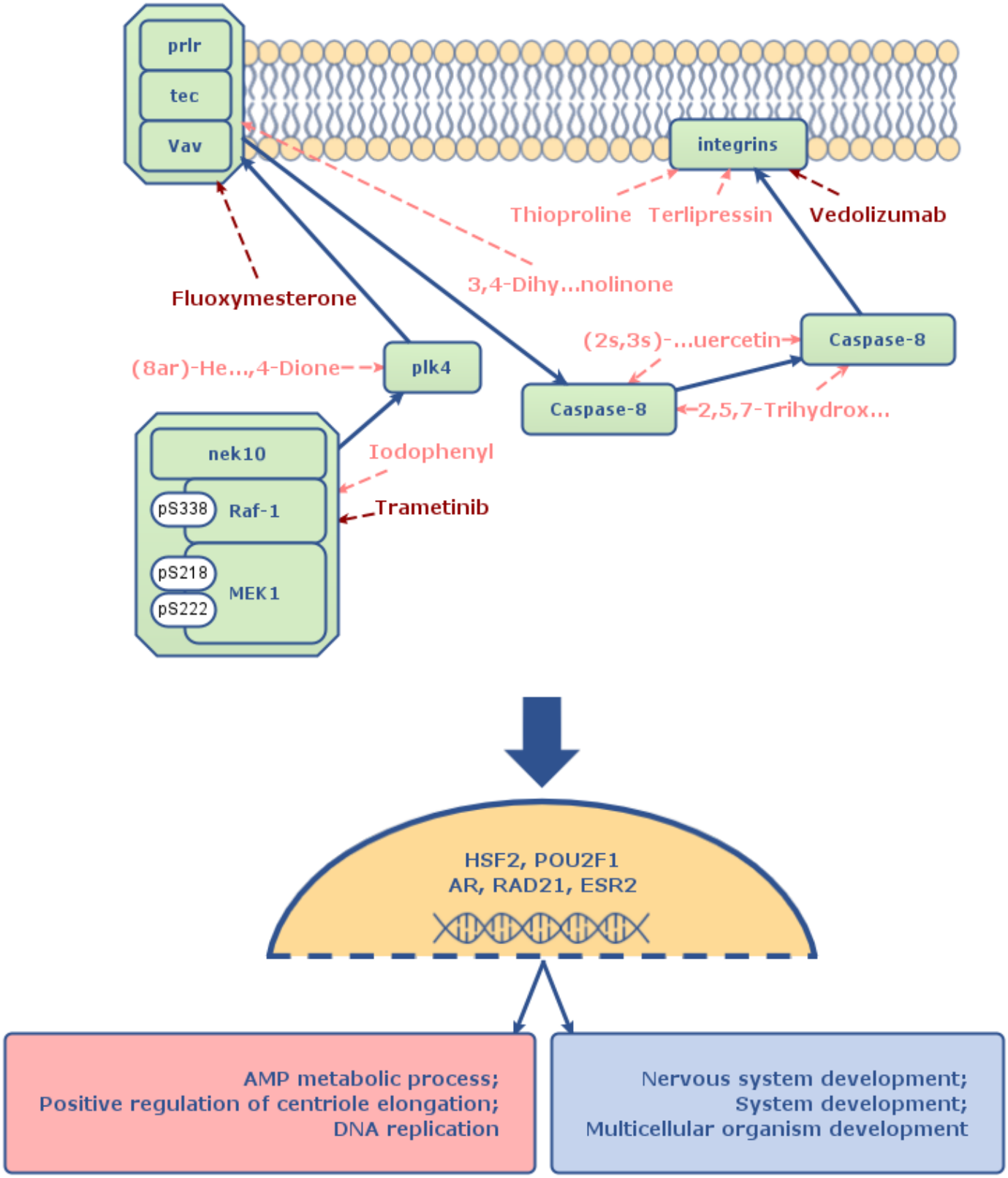
See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
PLK4	polo like kinase 4	0.36	0.23	437
NEK10	NIMA related kinase 10	1.58	0.2	490
LRRK2	leucine rich repeat kinase 2	3.25	0.32	494
ITGA6	integrin subunit alpha 6	6.21	0.22	496
ITGA4	integrin subunit alpha 4	10.3	0.22	496
ITGA1	integrin subunit alpha 1	6.21	0.22	496

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:

- Caspase-8
- integrins
- nek10:Raf-1{pS338}:MEK1{pS218}{pS222}
- plk4
- prlr:tec:Vav
- Caspase-8

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: Terlipressin, 2,5,7-Trihydroxynaphthoquinone, Thioproline, (2s,3s)-Trans-Dihydroquercetin, Vedolizumab, Iodophenyl, 3,4-Dihydro-5-Methyl-Isoquinolinone, Trametinib, Fluoxymesterone and (8ar)-Hexahydropyrrolo[1,2-a]Pyrazine-1,4-Dione, 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 12. 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)
Pioglitazone	PPARG	79	2	Angina Pectoris, Angina, Unstable, Apnea, Ascites, Ataxia, Ataxia Telangiectasia, Atherosclerosis...	small molecule, approved, investigational
ONO-2506	S100B	149	2	This drug was not tested on Phase 4 clinical trials yet. See full table for more details.	small molecule, investigational
Repaglinide	PPARG	152	1	Diabetes Mellitus, Diabetes Mellitus, Type 2	small molecule, approved, investigational
CEP-1347	MAPK12	197	5	This drug was not tested on Phase 4 clinical trials yet. See full table for more details.	small molecule, investigational
Hydrocortisone	NR3C1	271	3	Adrenal Insufficiency, Asthma, Bites and Stings, Burns, Candidiasis, Cicatrix, Cicatrix, Hypertrophic...	small molecule, approved

Repurposing drugs

Table 13. 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)
Peginterferon alfa-2a	IFNAR1, IFNAR2	22	HIV Infections, Hemophilia A, Hepatitis, Hepatitis B, Hepatitis B, Chronic, Hepatitis C, Hepatitis C, Chronic...	biotech, approved, investigational
Peginterferon alfa-2b	IFNAR1, IFNAR2	22	Hepatitis, Hepatitis B, Hepatitis B, Chronic, Hepatitis C, Hepatitis C, Chronic, Hepatitis, Chronic	biotech, approved
Interferon beta-1a	IFNAR1, IFNAR2	22	Brain Abscess, Multiple Sclerosis, Multiple Sclerosis, Relapsing-Remitting	biotech, approved, investigational
Interferon beta-1b	IFNAR1, IFNAR2	22	Brain Abscess, Multiple Sclerosis, Multiple Sclerosis, Relapsing-Remitting	biotech, approved
Interferon alfacon-1	IFNAR1, IFNAR2	22	Hepatitis, Hepatitis C	biotech, approved

Table 14. 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
Lipoic Acid	PTPN3, PTPRO, PTPRJ, EPM2A, PTPN2, PTPN13, DUSP16...	70	1.19
Tiludronate	IL7, PTPN3, PTPRO, PTPRJ, EPM2A, PTPN2, PTPN13...	90	0.87
3-(Phosphonomethyl)Pyridine-2-Carboxylic Acid	PTPN3, PTPRO, PTPRJ, EPM2A, PTPN2, PTPN13, DUSP16...	115	1.22
[[N-(Benzyloxycarbonyl)Amino]Methyl]Phosphate	PTPN3, PTPRO, PTPRJ, EPM2A, PTPN2, PTPN13, DUSP16...	118	0.85
D-Myo-Inositol-Hexasulphate	CDC25A, DUSP5, GGPS1, EPM2A, DUSP10, CDC14B, DUSP16...	150	0.35

Table 15. 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, SENP6, EPM2A, CASP8, MAPK6, DUSP16, PTP4A1...	220	0.84
3-(4-HYDROXY-3-METHOXYPHENYL)-2-PROPENOIC ACID	MAPK14, MAPK10, IL7, TLR4, MAPK12, CASP8, MAPK11...	231	0.35
Isoformononetin	MAPK14, MAPK10, MAPK12, CASP8, HIF1A, MAPK11, CASP3...	239	0.34
Thioproline	IL7, ITGA6, ITGA4, ITGB1, ITGAV, ITGA2, ITGA1	248	0.34
Sodium Tetradecyl Sulfate	CDC25A, DUSP5, GGPS1, EPM2A, DUSP10, CDC14B, DUSP16...	255	0.29

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Pioglitazone, Peginterferon alfa-2a, Lipoic Acid and 2,5,7-Trihydroxynaphthoquinone. These drugs were selected for acting on the following targets: PPARG, IFNAR1, UBASH3B and SENP6, 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 data set that contains *transcriptomics* data. The study is done in the context of *Parkinson Disease*. 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:

Pioglitazone, Peginterferon alfa-2a, Lipoic Acid and 2,5,7-Trihydroxynaphthoquinone

These drugs were selected for acting on the following targets: PPARG, IFNAR1, UBASH3B and SENP6, 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:

Caspase-8, integrins, nek10:Raf-1{pS338}:MEK1{pS218}{pS222}, plk4, prlr:tec:Vav and Caspase-8

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: Terlipressin, 2,5,7-Trihydroxynaphthoquinone, Thioproline, (2s,3s)-Trans-Dihydroquercetin, Vedolizumab, Iodophenyl, 3,4-Dihydro-5-Methyl-Isoquinolinone, Trametinib, Fluoxymesterone and (8ar)-Hexahydropyrrolo[1,2-a]Pyrazine-1,4-Dione. 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.

- Caspase-8
- integrins
- nek10:Raf-1{pS338}:MEK1{pS218}{pS222}
- plk4
- prlr:tec:Vav
- Caspase-8

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 2020.3 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2020.3 (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 2020.3 (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

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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 noRA_Dox vs. noRA_noDox).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in noRA_Dox vs. noRA_noDox).
5. Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.

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