NTRK2 and ODC1 are promising druggable targets for treating Squamous Cell Carcinoma that control activity of MEF2D, JUN and MAZ transcription factors on promoters of differentially expressed genes

Demo User
geneXplain GmbH
info@genexplain.com
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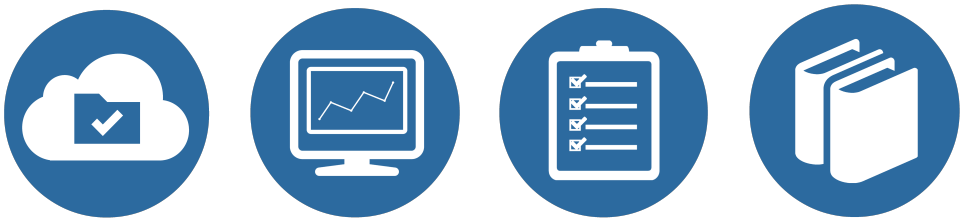
Genome Enhancer release 3.3 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2023.2)



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 *Squamous Cell Carcinoma*. 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: MEF2D, JUN, SMAD3, MAZ, FOSB and SMAD4. The subsequent network analysis suggested

- ornithine decarboxylase
- trkB

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: Erlotinib, seliciclib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE.

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
SRR349741.fastq	Transcriptomics
SRR349742.fastq	Transcriptomics
SRR349748.fastq	Transcriptomics
SRR349749.fastq	Transcriptomics

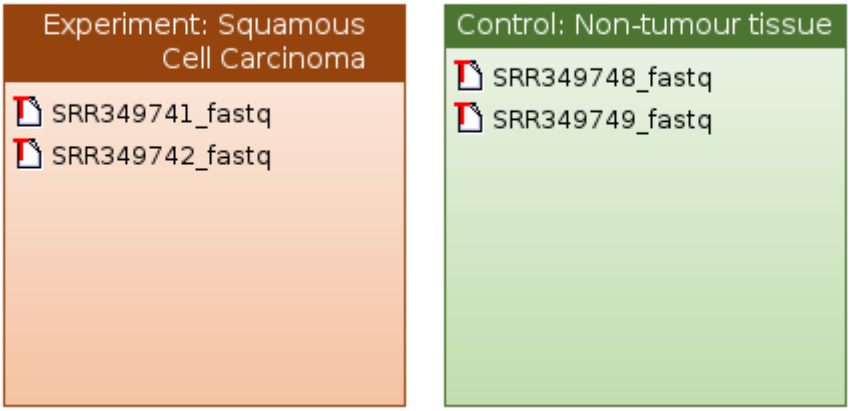


Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

3. Results

We have compared the following conditions: Experiment: Squamous Cell Carcinoma *versus* Control: Non-tumour tissue.

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: "Experiment: Squamous Cell Carcinoma" with "Control: Non-tumour tissue". 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 1955 upregulated genes (LogFC>0.1) out of which 768 genes were found as significantly upregulated (p-value<0.1) and 1739 downregulated genes (LogFC<-0.1) out of which 650 genes were significantly downregulated (p-value<0.1). See tables below for the top significantly up- and downregulated genes. Below we call **target genes** the full list of up- and downregulated genes revealed in our analysis (see tables in Supplementary section).

*Table 2. Top ten significant **up-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.*
See full table →

ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000115758	ODC1	ornithine decarboxylase 1	6.73	10.37	6.78E-9	6.85E-7
ENSG00000148053	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	9.33	1.47E-9	1.99E-7
ENSG00000113140	SPARC	secreted protein acidic and cysteine rich	5.74	10.75	1.44E-7	9.8E-6
ENSG00000163359	COL6A3	collagen type VI alpha 3 chain	5.19	9.2	1.54E-5	4.36E-4
ENSG00000120708	TGFBI	transforming growth factor beta induced	4.81	8.83	1.53E-9	2.01E-7
ENSG00000134871	COL4A2	collagen type IV alpha 2 chain	4.69	8.02	9.35E-10	1.36E-7
ENSG00000186340	THBS2	thrombospondin 2	4.67	8.54	6.35E-5	1.34E-3
ENSG00000146648	EGFR	epidermal growth factor receptor	4.44	9.65	3.25E-4	4.84E-3
ENSG00000145824	CXCL14	C-X-C motif chemokine ligand 14	4.43	8.61	2.44E-5	6.33E-4
ENSG00000187134	AKR1C1	aldo-keto reductase family 1 member C1	4.41	9.04	1.06E-10	2.88E-8

*Table 3. Top ten significant **down-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.*
See full table →

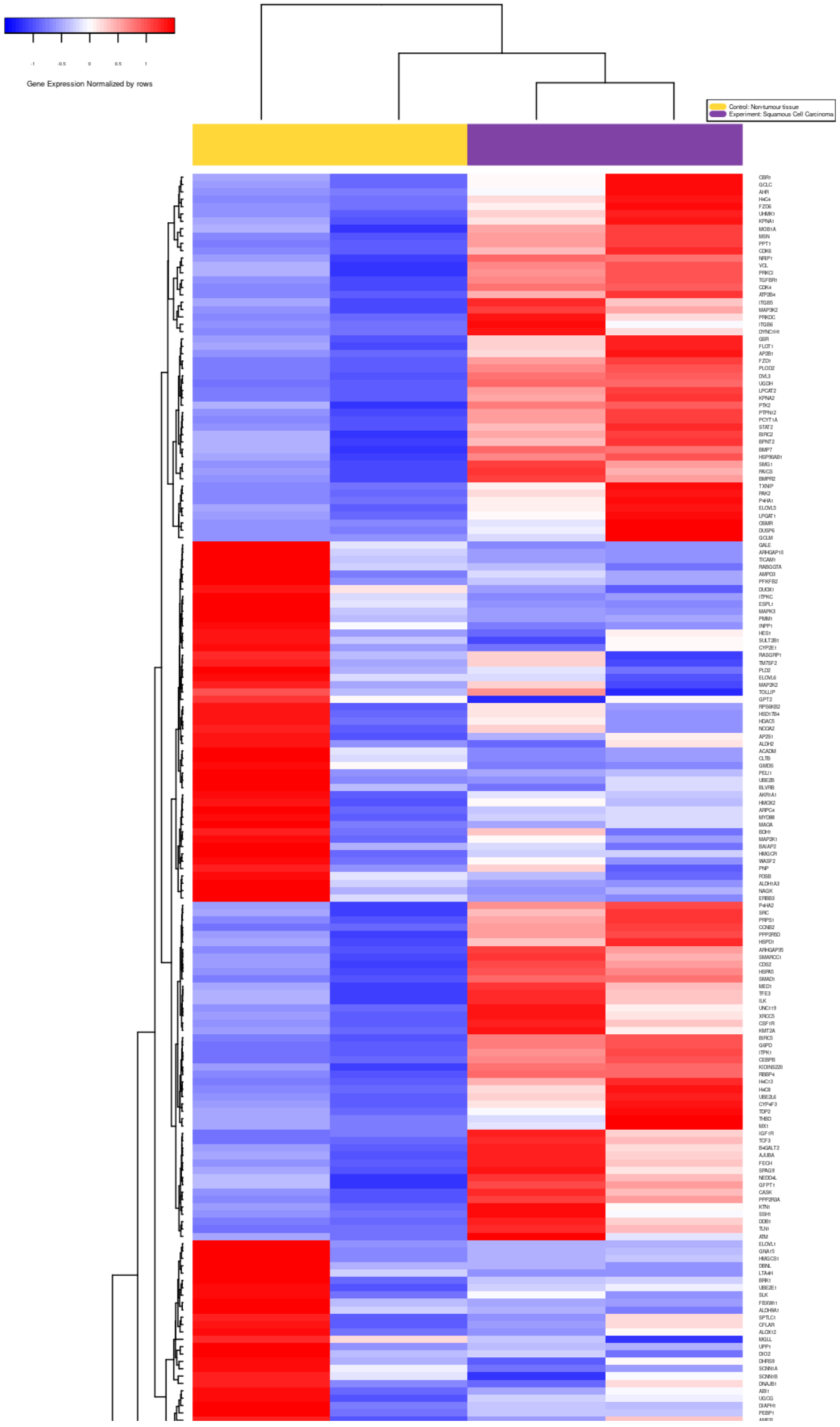
ID	Gene symbol	Gene description	logFC	logCPM	PValue	FDR
ENSG00000136155	SCEL	sciellin	-7.72	11.12	2.73E-15	5.38E-12
ENSG00000163209	SPRR3	small proline rich protein 3	-6.69	14.45	8.44E-4	1.1E-2
ENSG00000143369	ECM1	extracellular matrix protein 1	-6.38	11.04	4.35E-10	7.45E-8
ENSG00000189334	S100A14	S100 calcium binding protein A14	-6.37	10.46	1.1E-10	2.88E-8
ENSG00000229732		novel transcript	-6.27	12.97	4.93E-12	2.42E-9
ENSG00000086548	CEACAM6	CEA cell adhesion molecule 6	-6.2	10.31	5.18E-14	4.37E-11
ENSG00000171401	KRT13	keratin 13	-6.15	14.93	8.06E-11	2.44E-8
ENSG00000087128	TMPRSS11E	transmembrane serine protease 11E	-5.98	10.11	6.26E-9	6.48E-7
ENSG00000197632	SERPINB2	serpin family B member 2	-5.86	8.73	5.56E-14	4.37E-11
ENSG00000165272	AQP3	aquaporin 3 (Gill blood group)	-5.81	11.35	5.75E-5	1.23E-3

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 Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue

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: Non-tumour tissue
Experiment: Squamous Cell Carcinoma
CBR1
GCLC
AHR
H4C4
FZD6
UHMK1
KPNA1
MOB1A
MSN
PPT1
CDK6
NRIP1
VCL
PRKCI
TGFBR1
CDK4
ATP2B4
ITGB5
MAP3K2
PRKDC
ITGB6
DYNC1H1
GSR
FLOT1
AP2B1
FZD1
PLOD2
DVL3
UGDH
LPCAT2
KPNA2
PTK2
PTPN12
PCYT1A
STAT2
BIRC2
BPNT2
BMP7
HSP90AB1
SMG1
RAC3
BMPR2
TXNIP
PAK2
P4HA1
ELOVL5
LPGAT1
OSMR
DUSP6
GCLM
GALE
ARHGAP10
TICAM1
RABGGTA
AMPD3
PFKFB2
DUOX1
ITPKC
ESPL1
MAPK3
PMM1
INPP1
HES1
SULT2B1
CYP2E1
RASGRP1
TMTSF2
PLD2
ELOVL6
MAP2K2
TOLLIP
GPT2
RPS6KB2
HSD17B4
HDAC5
NCOA2
AP2S1
ALDH2
ACADM
CLTB
GMDS
PELI1
UBE2B
BLVRB
AKR1A1
HMOX2
AXPC4
MYD88
MAGA
BDH1
MAP2K1
BAIAP2
HMGCR
WASF2
PNP
FOSB
ALDH1A3
NAGK
EPB83
P4HA2
SRC
PRPS1
CCNB2
PPP2R5D
HSPD1
ARHGAP35
SMARCC1
CDS2
HSPA5
SMAD1
MED1
TFE3
ILK
UNC119
XRCC5
CSF1R
KMT2A
BIRC5
G6PD
ITPK1
CEBPB
KIDINS220
RBBP4
H4C13
H4C8
UBE2L6
CYP4F3
TDP2
THBD
MX1
IGF1R
TCF3
B4GALT2
AJUBA
FECH
SPNS9
NEDD4L
GFPT1
CASK
PPP2R3A
KTN1
SSH1
DDB1
TLN1
ATM
ELOVL1
GNA15
HMGCS1
DBNL
LTA4H
BRK1
UBE2E1
SLK
FBXW11
ALDH9A1
SPTLC1
CFLAR
ALOX12
MGLL
UPP1
DIO2
DHRS9
SCNN1A
SCNN1B
DNAJB1
ABI1
UGCG
DIAPH1
PEBP1
AMER

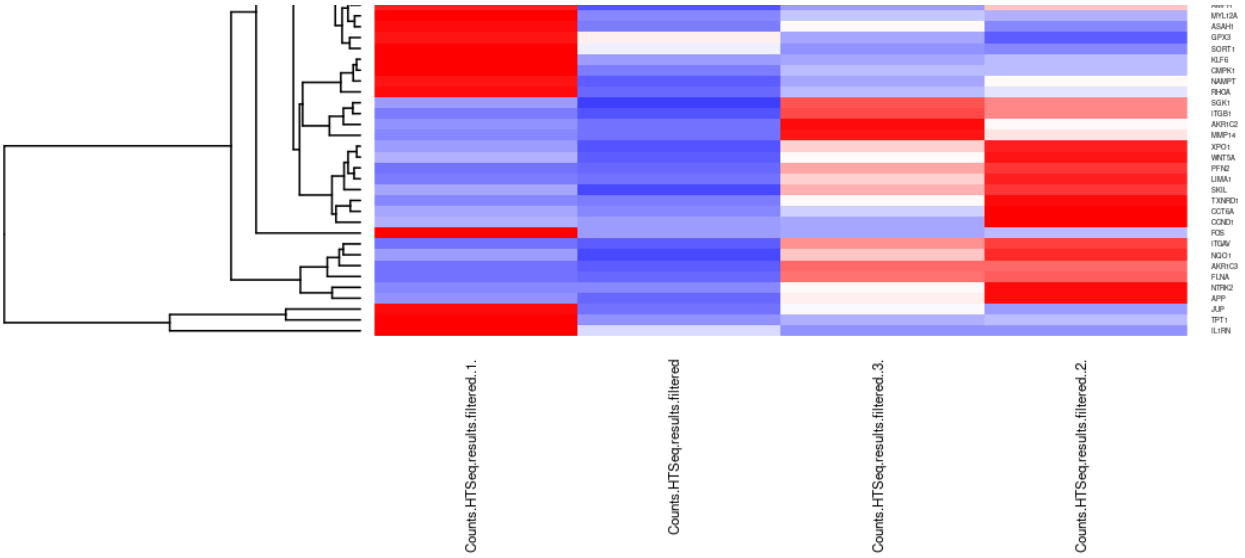


Figure 2. Heatmap of genes enriched in Transpath categories. The colored bar at the top shows the types of the samples according to the legend in the upper right corner.

See full diagram →

Up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue:

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

GO (biological process)

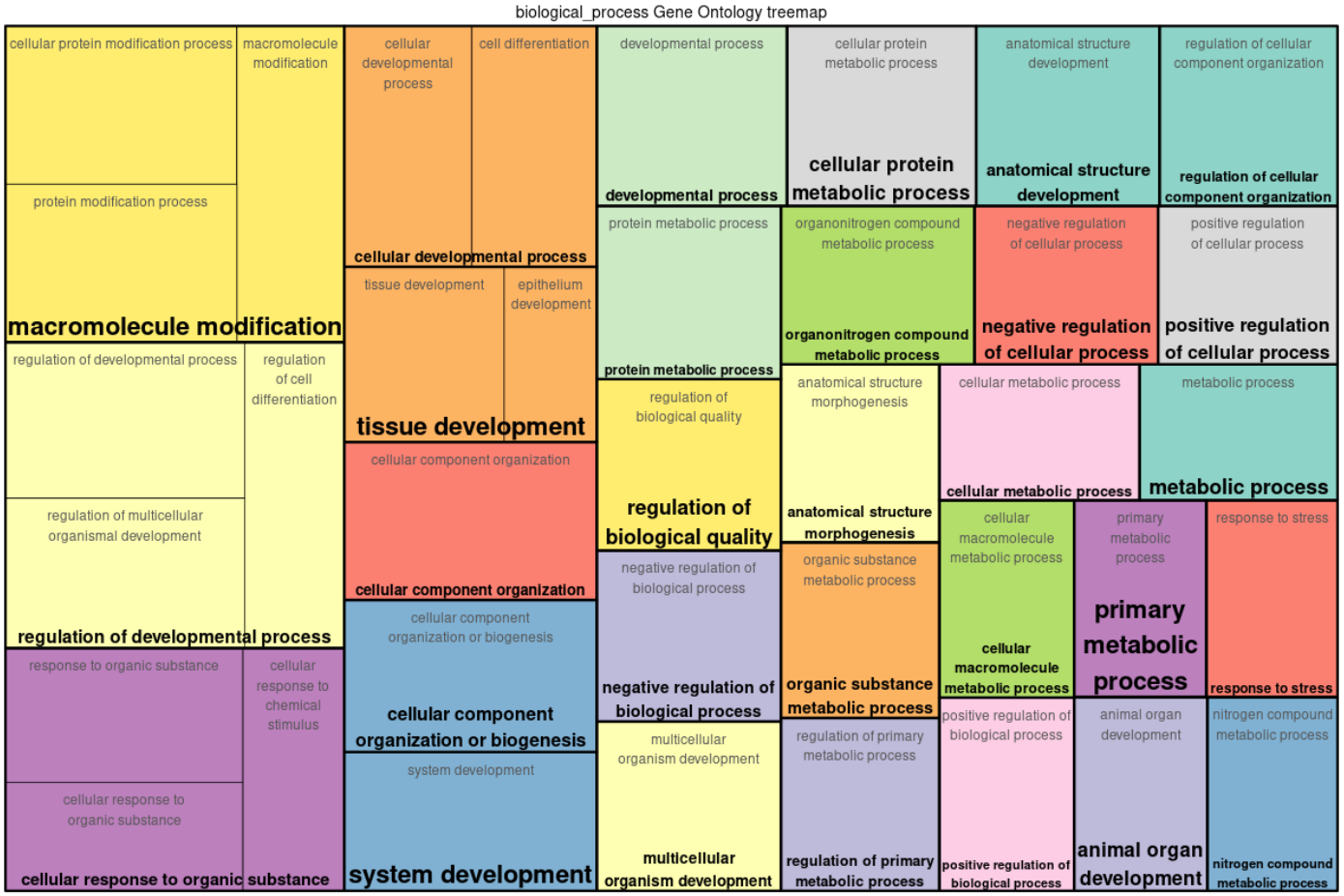


Figure 3. Enriched GO (biological process) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

TRANSPATH® Pathways (2023.2)

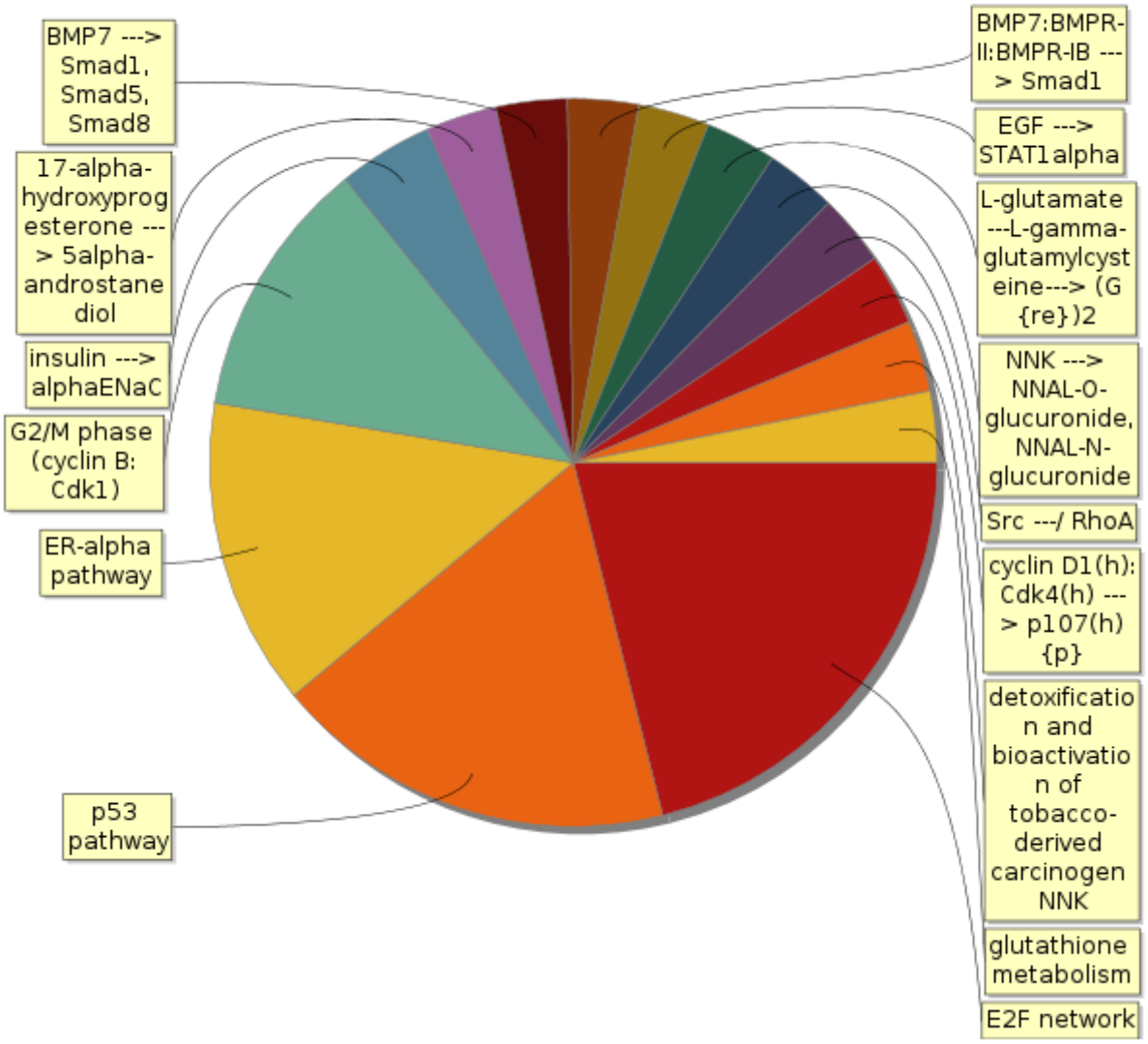


Figure 4. Enriched TRANSPATH® Pathways (2023.2) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

HumanPSD(TM) disease (2023.2)

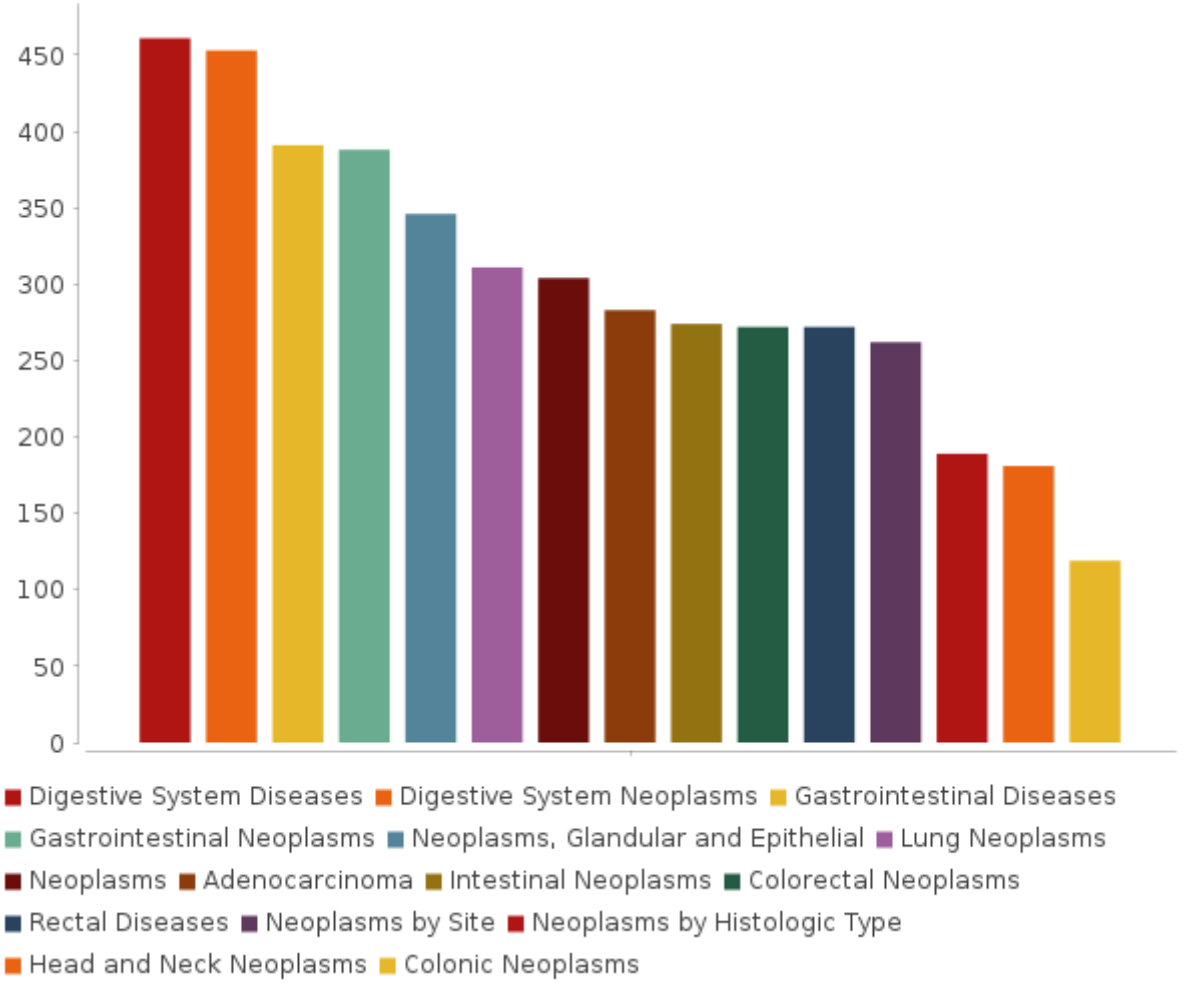


Figure 5. Enriched HumanPSD(TM) disease (2023.2) of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.

Full classification →

Down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue:

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

GO (biological process)

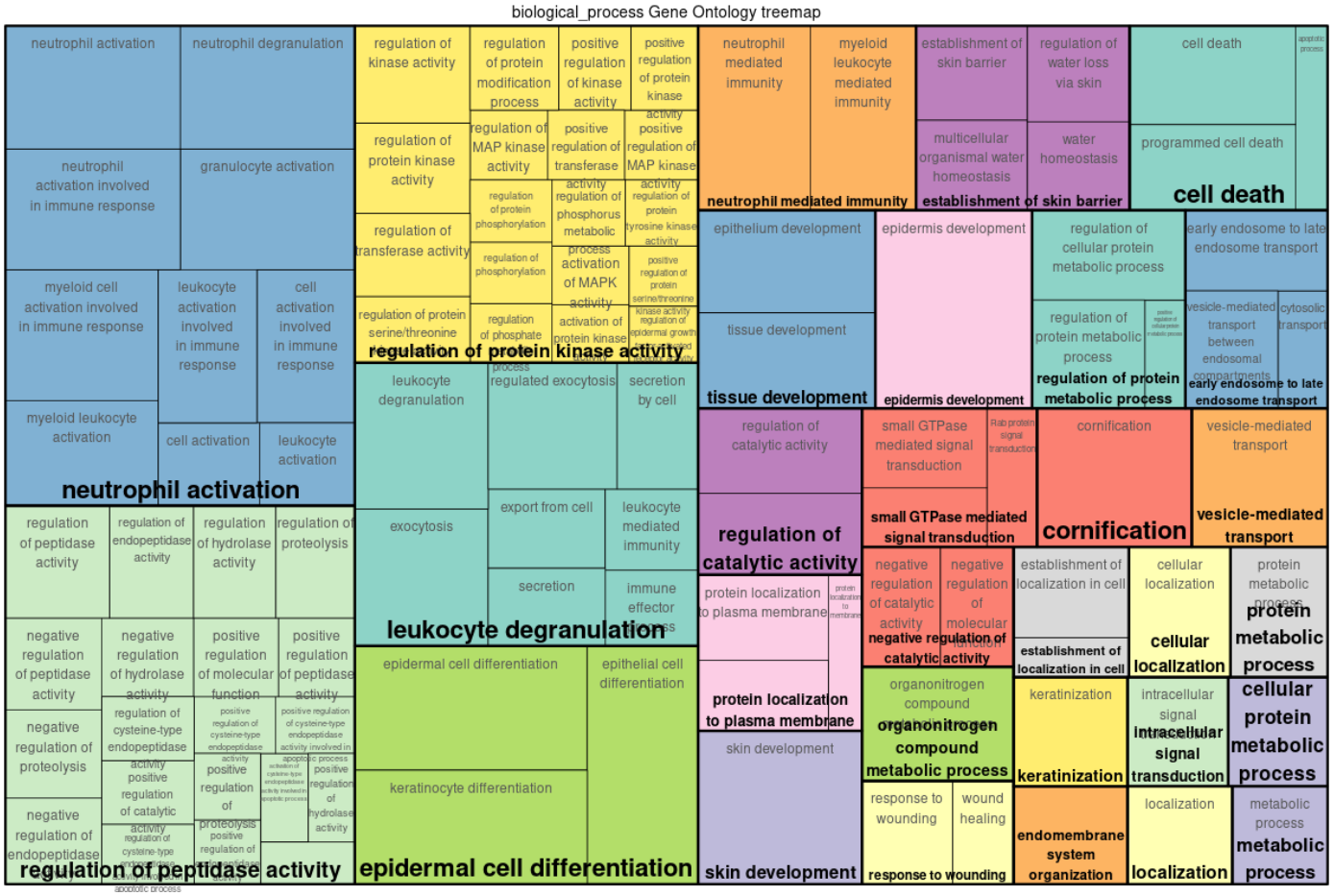


Figure 6. Enriched GO (biological process) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

TRANSPATH® Pathways (2023.2)

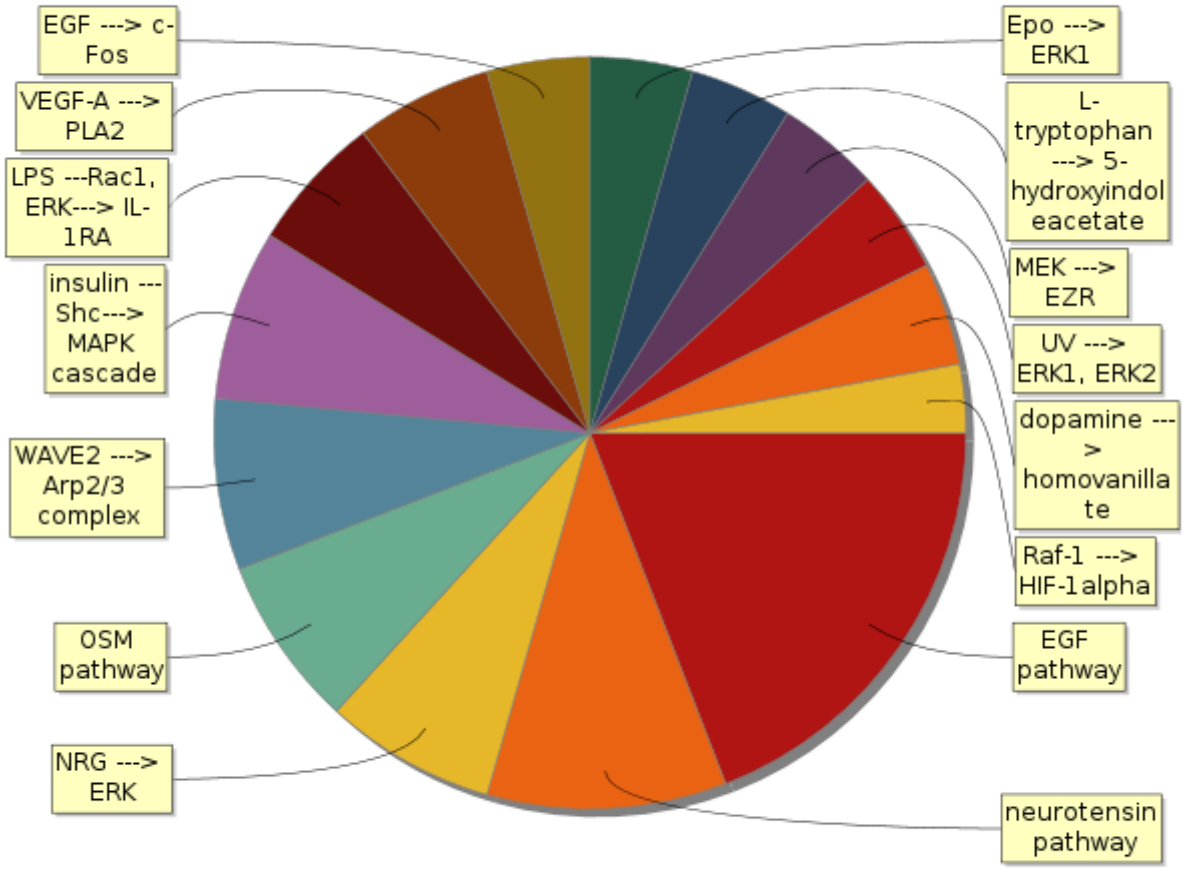


Figure 7. Enriched TRANSPATH® Pathways (2023.2) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

Full classification →

HumanPSD(TM) disease (2023.2)

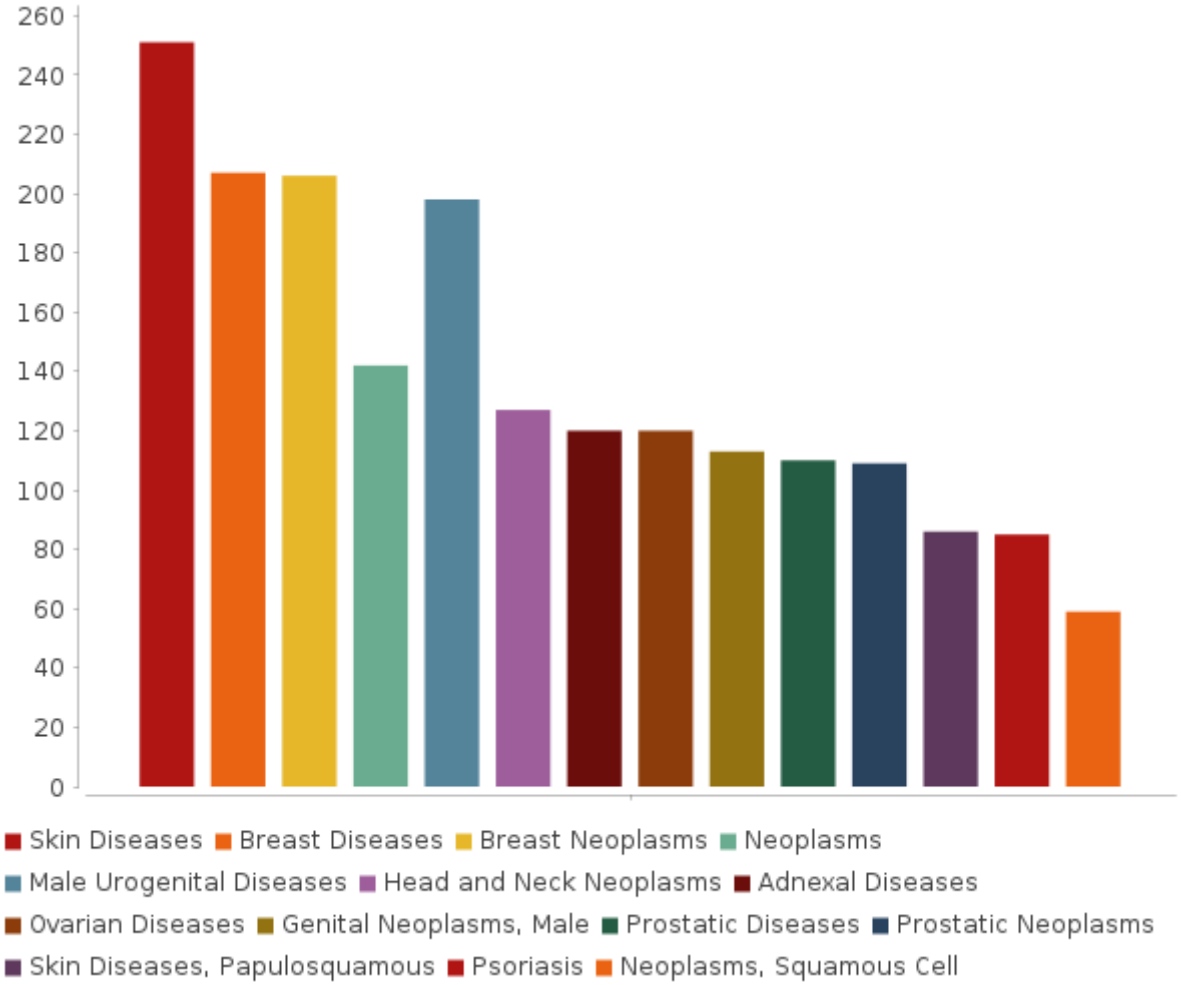


Figure 8. Enriched HumanPSD(TM) disease (2023.2) of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.

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].

In the current work, we use the Genomics data from the "Yes VCF track" track to predict positions of potential ***enhancers*** where the observed sequence variations may influence the gene expression in the pathology under study. We scan 5kb flanking regions and the body of all genes caring the variations, with a sliding window of 1100bp size and find the position of the window with the maximal sum of the mutation weights, where we then perform the search for potential condition-specific enhancers (CMA model search).

We analyzed mutations that were revealed in the potential enhancers located upstream, downstream or inside the ***target genes*** (see Table 4). We identified 638 mutations potentially affecting gene regulation. Table 5 shows the following lists of PWMs whose sites were lost or gained due to these mutations. Weighting of mutations was done in respect to the significance of the change in TF affinity binding to the sequence. Mutations that maximally affected the change of binding affinity received higher weights. These PWMs were put in focus of the CMA algorithm that constructs the model of the enhancers by specifying combinations of TF motifs (see more details of the algorithm in the Methods section).

Table 4. Mutations revealed in Experiment: Squamous Cell Carcinoma versus Control: Non-tumour tissue

See full table →

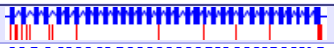
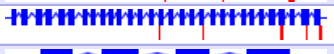
ID	Gene symbol	Gene schematic representation	Number of variations
ENSG00000146648	EGFR		21
ENSG00000083857	FAT1		16
ENSG00000134871	COL4A2		13
ENSG00000186340	THBS2		10
ENSG00000226445	ENSG00000226445		9
ENSG00000145012	LPP		8
ENSG00000114999	TTL		7
ENSG00000142173	COL6A2		7
ENSG00000152291	TGOLN2		7
ENSG00000157214	STEAP2		7

Table 5. PWMs whose sites were lost or gained due to mutations in Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue

See full table →

ID	P-value (gains)	P-value (losses)	yesCount (gains)	yesCount (losses)
V$EGR1_07	4.62E-2	1.48E-24	5	1134
V$E2F7_04	3.9E-2	5.96E-23	11	744
V$GLI2_05	2.5E-2	1.36E-22	11	2807
V$E2F3_05	1.58E-2	3.85E-25	27	1467
V$E2F1_Q4_01	1.5E-2	1.98E-27	11	1490
V$TFCP2_06	2.67E-3	2.13E-16	7	3313
V$RUNX3_01	5.83E-6	3.04E-24	151	1895
V$E2F1_05	3.16E-7	6.77E-27	39	1042
V$TEF_05	2.04E-7	1.43E-18	452	538
V$E2F7_01	2.69E-11	5.76E-16	73	153
V$MEIS1ELF1_01	2.29E-11	1.37E-16	2061	1805
V$TFDP1_03	1.12E-12	6.17E-24	275	1398
V$GCM1_08	5.16E-18		852	
V$OSX_Q3	5.11E-18	4.62E-2	352	5
V$GLI1_Q3	1.34E-19		833	
V$ZNF282_03	9.82E-20		803	
V$MECP2_02	3.65E-20	1.39E-3	738	39
V$SP1_09	3.12E-20	4.61E-2	342	4
V$E2F1DP2_01	1.1E-20	1.22E-16	2155	2222
V$E2F1EOMES_02	8.12E-21	5.89E-4	705	366

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

Enhancer model potentially involved in regulation of target genes (up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).

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 in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

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$OSX_Q3 0.99; N=2 | V$SMAD1_Q6 0.97; N=1 | V$SMAD3_04 0.97; N=3 | V$IRF4_Q6 0.98; N=3 | V$MEF2D_06 0.82; N=3 | V$JUNFRA1_01 0.89; N=2 | V$GATA2_15 0.99; N=2

Module width: 127

Module 2:

V$OSX_Q3 1.00; N=3 | V$SMAD1_Q6 0.97; N=2 | V$NR3C2_03 0.91; N=3 | V$SF1_04 0.95; N=3 | V$SOX2_Q6 0.96; N=3 | V$HOXA9_05 0.93; N=2 | V$CUX1FOXI1_01 0.85; N=2

Module width: 123

Model score (-p*log10(pval)): 13.44
Wilcoxon p-value (pval): 2.16e-30
Penalty (p): 0.453
Average yes-set score: 3.48
Average no-set score: 2.06
AUC: 0.74
Separation point: 2.83
False-positive: 27.51%
False-negative: 31.44%

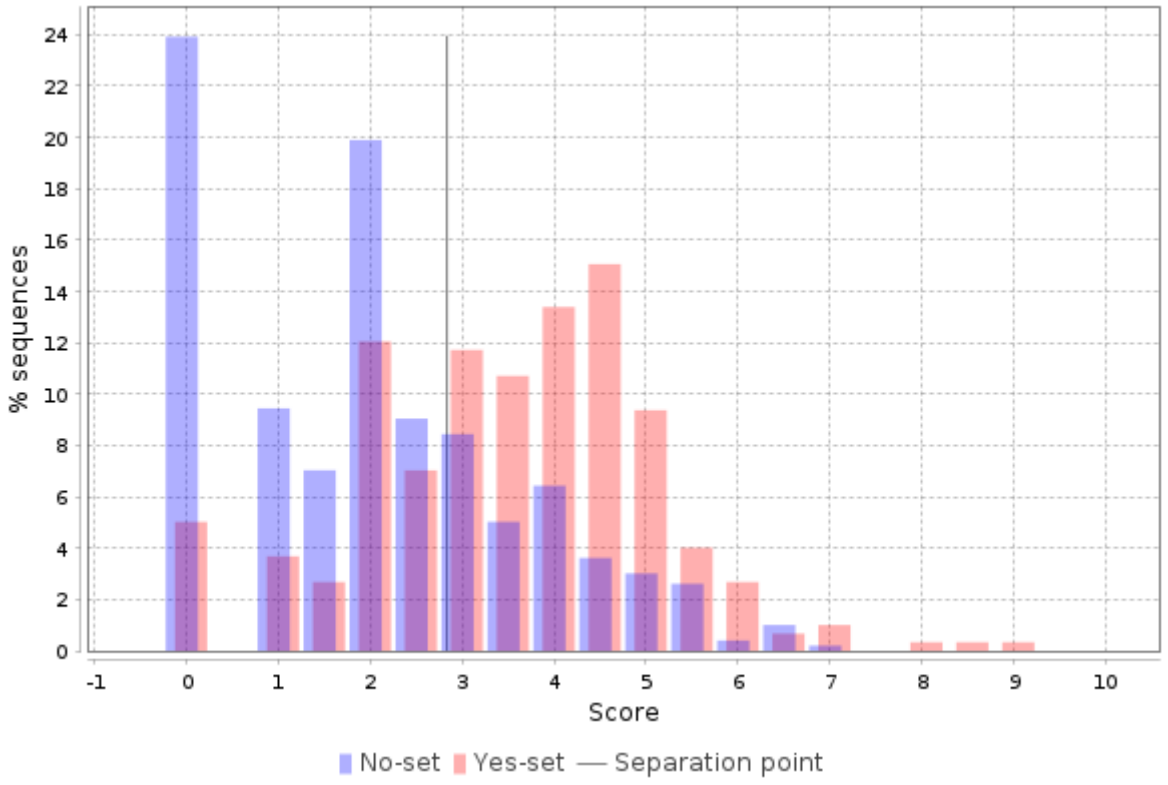


Table 6. List of top ten up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*
See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000144452	ABCA12	ATP binding cassette subfamily A member 12	9.06	MEF-2D(h), IRF-4(h), Fra-1(h),c-Jun(h), Sp7(h), FTZ-F1(h), MR(h), SMAD1(h)...
ENSG00000101350	KIF3B	kinesin family member 3B	8.3	IRF-4(h), FTZ-F1(h), MEF-2D(h), SOX-2(h), SMAD3(h), SMAD1(h), CUX-1(h),FOXI1(h)...
ENSG00000138078	PREPL	prolyl endopeptidase like	7.93	SMAD1(h), MR(h), MEF-2D(h), IRF-4(h)
ENSG00000001084	GCLC	glutamate-cysteine ligase catalytic subunit	7.87	Fra-1(h),c-Jun(h), Sp7(h), GATA-2(h), MR(h), FTZ-F1(h), SOX-2(h), SMAD1(h)...
ENSG00000053254	FOXN3	forkhead box N3	7.42	SMAD1(h), MR(h), CUX-1(h),FOXI1(h), MEF-2D(h), IRF-4(h)
ENSG00000102580	DNAJC3	DnaJ heat shock protein family (Hsp40) member C3	7.4	CUX-1(h),FOXI1(h), MR(h), MEF-2D(h), IRF-4(h), SMAD3(h), GATA-2(h), Fra-1(h),c-Jun(h)
ENSG00000166949	SMAD3	SMAD family member 3	7.32	SMAD3(h), SMAD1(h), MR(h), Sp7(h), MEF-2D(h)
ENSG00000100320	RBFOX2	RNA binding fox-1 homolog 2	7.19	Sp7(h), SMAD1(h), SMAD3(h), MR(h)
ENSG00000111371	SLC38A1	solute carrier family 38 member 1	7.16	SOX-2(h), IRF-4(h), MR(h), MEF-2D(h), SMAD3(h)
ENSG00000188559	RALGAPA2	Ral GTPase activating protein catalytic subunit alpha 2	7.09	SOX-2(h), MR(h), SMAD3(h), MEF-2D(h), IRF-4(h), Fra-1(h),c-Jun(h)

Enhancer model potentially involved in regulation of target genes (down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).

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 in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue.

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$ZNF462_01	V$RELBP50_Q3	V$FOSBJUND_01	V$MAZ_Q6	V$WT1_Q6_02
0.92; N=2	0.86; N=2	0.88; N=3	0.97; N=1	0.95; N=2

Module width: 144

Module 2:

V$GLI1_Q3	V$FLI1_09	V$AREB6_02	V$SMAD4_Q6
0.91; N=2	0.83; N=2	0.96; N=2	0.82; N=2

Module width: 144

Model score (-p*log10(pval)): 15.24
Wilcoxon p-value (pval): 3.42e-30
Penalty (p): 0.517
Average yes-set score: 6.92
Average no-set score: 5.51
AUC: 0.74
Separation point: 6.65
False-positive: 22.29%
False-negative: 39.46%

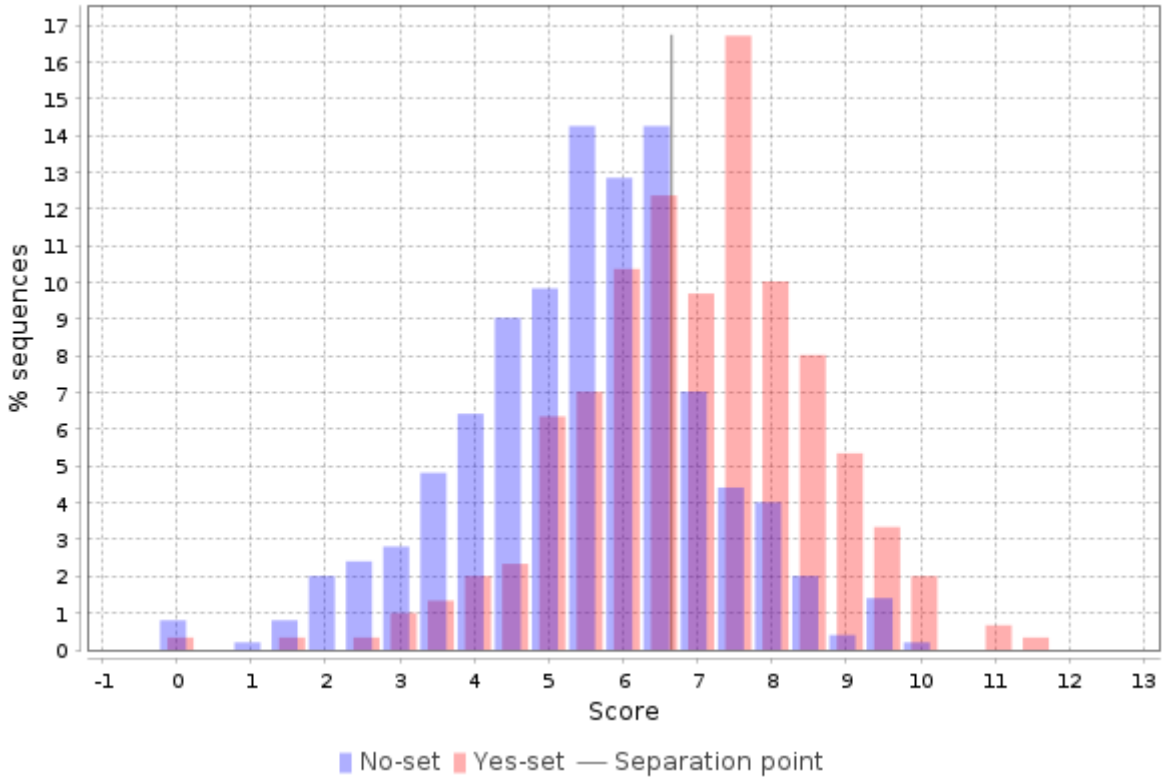


Table 7. List of top ten down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue with identified enhancers in their regulatory regions. ***CMA score*** *- the score of the CMA model of the enhancer identified in the regulatory region.*

See full table →

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000160685	ZBTB7B	zinc finger and BTB domain containing 7B	12.63	WT1(h), FosB(h),JunD(h), ZNF462(h), MAZ(h), RelB(h), SMAD4(h), FLI-1(h)...
ENSG00000188505	NCCRP1	NCCRP1, F-box associated domain containing	11.53	FLI-1(h), ZEB1(h), GLI1(h), SMAD4(h), FosB(h),JunD(h), RelB(h), ZNF462(h)...
ENSG00000005001	PRSS22	serine protease 22	11.27	GLI1(h), ZEB1(h), SMAD4(h), FLI-1(h), MAZ(h), FosB(h),JunD(h), ZNF462(h)...
ENSG00000184828	ZBTB7C	zinc finger and BTB domain containing 7C	11.18	ZNF462(h), FosB(h),JunD(h), MAZ(h), FLI-1(h), GLI1(h), ZEB1(h), SMAD4(h)
ENSG00000065361	ERBB3	erb-b2 receptor tyrosine kinase 3	11.16	ZEB1(h), GLI1(h), FLI-1(h), SMAD4(h), ZNF462(h), WT1(h), MAZ(h)...
ENSG00000134107	BHLHE40	basic helix-loop-helix family member e40	10.95	ZEB1(h), RelB(h), ZNF462(h), FosB(h),JunD(h), MAZ(h), FLI-1(h), GLI1(h)...
ENSG00000119900	OGFRL1	opioid growth factor receptor like 1	10.79	RelB(h), FosB(h),JunD(h), ZNF462(h), FLI-1(h), WT1(h), MAZ(h), ZEB1(h)...
ENSG00000114166	KAT2B	lysine acetyltransferase 2B	10.76	FLI-1(h), SMAD4(h), GLI1(h), ZEB1(h), ZNF462(h), RelB(h), MAZ(h)...
ENSG00000166925	TSC22D4	TSC22 domain family member 4	10.38	GLI1(h), SMAD4(h), FLI-1(h), ZEB1(h), MAZ(h), ZNF462(h), WT1(h)...
ENSG00000201185	RNA5SP202	RNA, 5S ribosomal pseudogene 202	10.37	GLI1(h), SMAD4(h), ZNF462(h), FLI-1(h), ZEB1(h), RelB(h), FosB(h),JunD(h)

On the basis of the enhancer models we identified transcription factors potentially regulating the ***target genes*** of our interest. We found 14 and 10 transcription factors controlling expression of up- and down-regulated genes respectively (see Tables 8-9).

Table 8. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue). ***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 →

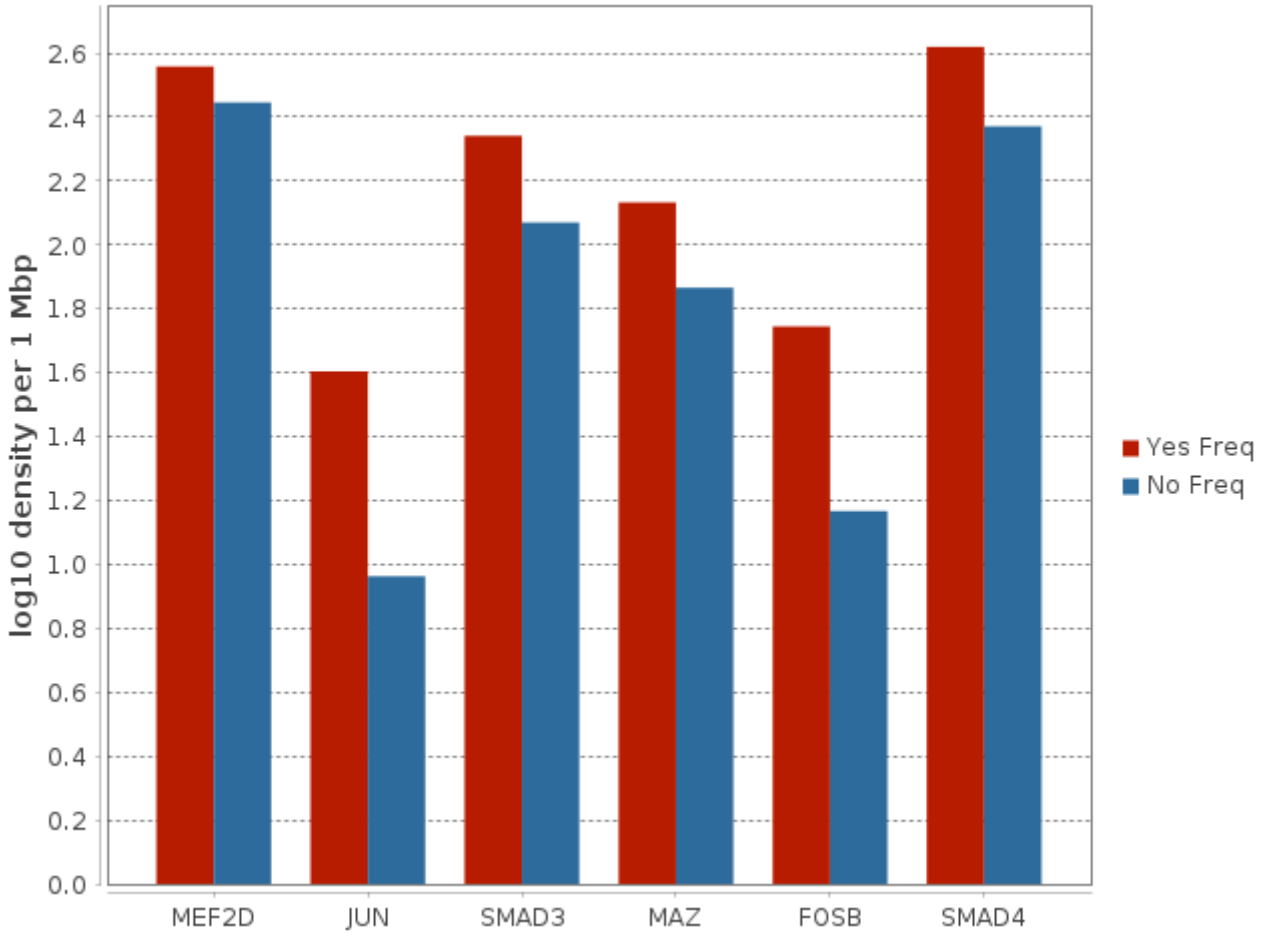
ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
MO000085555	MEF2D	myocyte enhancer factor 2D	4.09	1.3
MO000019469	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	3.82	4.38
MO000057832	SMAD3	SMAD family member 3	3.71	1.87
MO000019609	SMAD1	SMAD family member 1	3.5	1.53
MO000019620	NR5A1	nuclear receptor subfamily 5 group A member 1	3.11	1.77
MO000025684	FOSL1	FOS like 1, AP-1 transcription factor subunit	3.02	7.86
MO000021449	NR3C2	nuclear receptor subfamily 3 group C member 2	2.77	1.21
MO000028705	IRF4	interferon regulatory factor 4	2.75	1.99
MO000024708	CUX1	cut like homeobox 1	2.41	3.03
MO000032472	GATA2	GATA binding protein 2	2.41	6.17

*Table 9. Transcription factors of the predicted enhancer model potentially regulating the differentially expressed genes (down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue). **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
MO000105384	MAZ	MYC associated zinc finger protein	1.94	1.85
MO000082447	FOSB	FosB proto-oncogene, AP-1 transcription factor subunit	1.86	3.78
MO000020402	SMAD4	SMAD family member 4	1.82	1.77
MO000007834	JUND	JunD proto-oncogene, AP-1 transcription factor subunit	1.78	3.36
MO000139677	ZEB1	zinc finger E-box binding homeobox 1	1.74	2.17
MO000092587	ZNF462	zinc finger protein 462	1.68	1.29
MO000102040	WT1	WT1 transcription factor	1.41	2.17
MO000005191	FLI1	Fli-1 proto-oncogene, ETS transcription factor	1.39	1.56
MO000019372	RELB	RELB proto-oncogene, NF-kB subunit	1.25	1.5
MO000019117	GLI1	GLI family zinc finger 1	1.25	1.68

The following diagram represents the key transcription factors, which were predicted to be potentially regulating differentially expressed genes in the analyzed pathology: MEF2D, JUN, SMAD3, MAZ, FOSB and SMAD4.



3.4. Finding master regulators in networks

In the second step of the upstream analysis common regulators of the revealed TFs were identified. We identified 10 signaling proteins whose structure and function is highly damaged by the mutations (see Table 10).

Table 10. Signaling proteins whose structure and function are damaged by the mutations in Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue

See full table →

ID	Title	Mutation count	Consequence	Codons
MO000208420	GJB3(h)	2	stop_gained	tGg/tAg
MO000109306	PSMA4(h)	1	stop_lost	Tga/Cga
MO000119197	wolframin(h)	1	stop_gained	Caa/Taa
MO000144222	APT2(h)	1	stop_lost	Tag/Cag
MO000172130	c3orf1(h)	1	NMD_transcript_variant,stop_lost	tGa/tCa
MO000175986	oas2(h)	1	stop_lost	tAg/tGg
MO000189841	ZSWIM1(h)	1	stop_gained	tGg/tAg
MO000212738	EMC10(h)	1	stop_lost	taG/taT
MO000219203	PSMG1(h)	1	NMD_transcript_variant,stop_lost	Taa/Caa
MO000222634	TCP11L1(h)	1	NMD_transcript_variant,stop_gained	Cag/Tag

Top 10 mutated proteins for Experiment: Squamous Cell Carcinoma and Control: Non-tumour tissue were used in the algorithm of master regulator search as a list of nodes of the signal transduction network that are removed from the network during the search of master regulators (see more details about the algorithm in the Methods section). 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 11-12.

*Table 11. Master regulators that may govern the regulation of **up-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. **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
MO000032677	trkB(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	61
MO000256500	trkB-N-T1(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	62
MO000256495	trkB-T1(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	63
MO000256496	trkB-T-Shc(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	63
MO000256497	trkB-isoform4(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	63
MO000256498	trkB-isoform5(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	63
MO000256499	trkB-T-TK(h)	NTRK2	neurotrophic receptor tyrosine kinase 2	5.99	63
MO000090366	ornithine decarboxylase(h)	ODC1	ornithine decarboxylase 1	6.73	65
MO000329204	Cdk6(h):cyclinD3-isoform1(h)	CCND3, CDK6	cyclin D3, cyclin dependent kinase 6	2.69	142
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...	3.05	144

*Table 12. Master regulators that may govern the regulation of **down-regulated** genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. **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
MO0000019174	Eck(h)	EPHA2	EPH receptor A2	-3.32	45
MO0000056491	KAT2B(h)	KAT2B	lysine acetyltransferase 2B	-3.15	49
MO0000021356	EGFR(h){pY}	EGFR, ERBB2, ERBB3, ERBB4	epidermal growth factor receptor, erb-b2 receptor tyrosine kinase 2, erb-b2 receptor tyrosine kinase...	-2.6	60
MO0000033396	DUSP5(h)	DUSP5	dual specificity phosphatase 5	-4.8	83
MO0000031101	plk3(h)	PLK3	polo like kinase 3	-2.83	84
MO0000334531	Eck-isoform2(h)	EPHA2	EPH receptor A2	-3.32	109
MO0000137320	Eck-isoform1(h)	EPHA2	EPH receptor A2	-3.32	110
MO0000137304	DUSP5(h)	DUSP5	dual specificity phosphatase 5	-4.8	113
MO0000004672	ERK1(h)	MAPK3	mitogen-activated protein kinase 3	-2.26	129
MO0000003497	Csk(h)	CSK	C-terminal Src kinase	-1.57	144

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

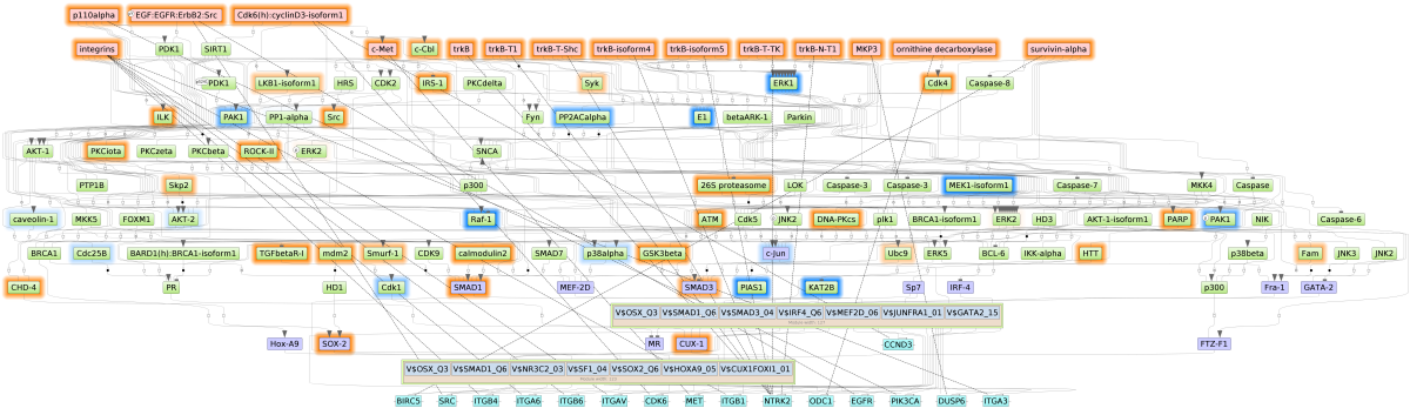


Figure 9. Diagram of intracellular regulatory signal transduction pathways of up-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate molecules,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

See full diagram →

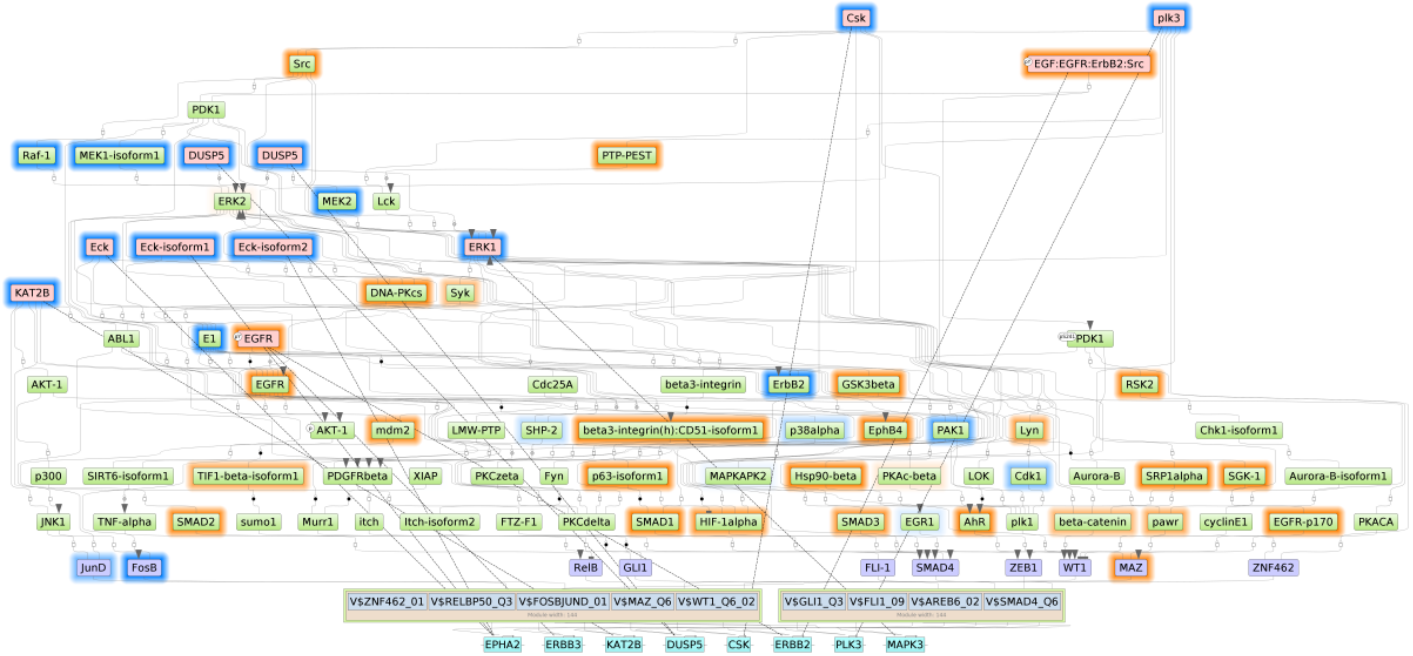


Figure 10. Diagram of intracellular regulatory signal transduction pathways of down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue. Master regulators are indicated by red rectangles, transcription factors are blue rectangles, and green rectangles are intermediate 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 Methods 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 13. 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
NTRK2	neurotrophic receptor tyrosine kinase 2	45	5.99	63
ODC1	ornithine decarboxylase 1	4	6.73	65
CCND3	cyclin D3	4	2.69	142
ITGA3	integrin subunit alpha 3	2	3.05	144
ITGB5	integrin subunit beta 5	2	3.05	144
ITGA6	integrin subunit alpha 6	1	3.05	144

*Table 14. 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.*

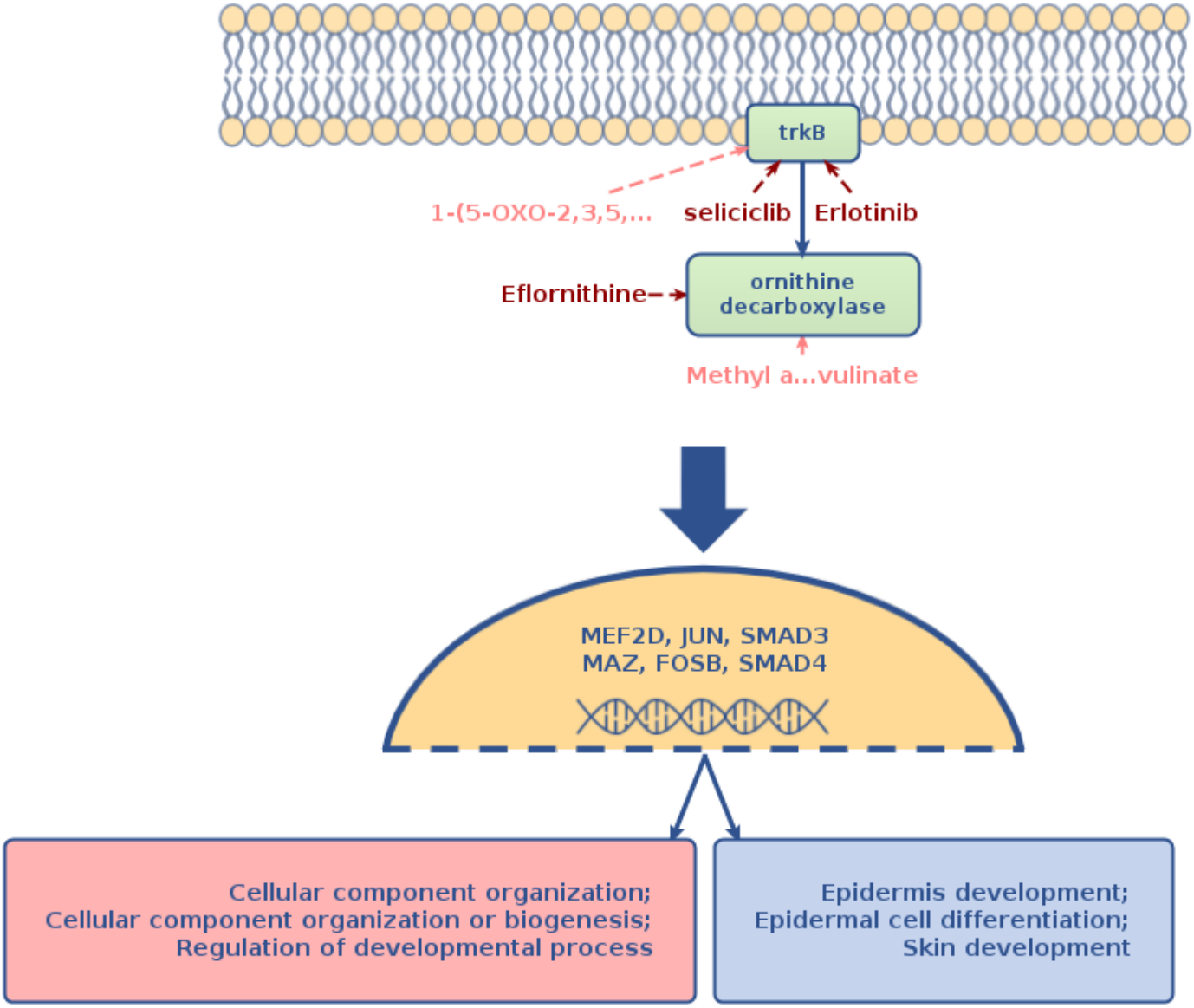
See full table →

Gene symbol	Gene Description	Druggability score	logFC	Total rank
NTRK2	neurotrophic receptor tyrosine kinase 2	12.4	5.99	63
ODC1	ornithine decarboxylase 1	0.41	6.73	65
CCND3	cyclin D3	14.31	2.69	142
ITGA3	integrin subunit alpha 3	6.21	3.05	144
ITGB5	integrin subunit beta 5	6.21	3.05	144
ITGA6	integrin subunit alpha 6	6.21	3.05	144

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:

- ornithine decarboxylase
- trkB

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: 1-(5-OXO-2,3,5,9B-TETRAHYDRO-1H-PYRROLO[2,1-A]ISOINDOL-9-YL)-3-(5-PYRROLIDIN-2-YL-1H-PYRAZOL-3-YL)-UREA, Erlotinib, seliciclib, Methyl aminolevulinate and Eflornithine, 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 the ranks sum based on the individual ranks of the following 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));
- Clinical validity score (applicable only for drugs predicted on the basis of literature curation in HumanPSD™ database (Tables 16 and 17), reflects the number of the highest clinical trials phase on which the drug was tested for any pathology).

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

Based on the Drug rank, a numerical value of Drug score was calculated, which reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. Drug score values belong to the range from 1 to 100 and are calculated as a quotient of maximum drug rank and the drug rank of the given drug multiplied by 100.

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

Drugs approved in clinical trials for Oncology

Table 15. Clinically approved (FDA, ENA, etc.) drugs for the studied pathology (most promising and clinically approved treatment candidates selected for the identified drug targets on the basis of literature curation in HumanPSD™ database)

See full table →

Name	Target names	Drug score	Disease activity score	Disease trial phase	Approved
Fluorouracil	PTPRC, BIRC5	52	11	Phase 4: Carcinoma, Squamous Cell, Bowen's Disease, Breast Neoplasms, Carcinoma, Carcinoma, Basal Cell, Colorectal Neoplasms, Digestive System Neoplasms, Gastrointestinal Neoplasms, Glaucoma, Head and Neck Neoplasms, Hypopigmentation, Intestinal Neoplasms, Keratosis, Keratosis, Actinic, Neoplasms, Neoplasms, Basal Cell, Neoplasms, Squamous Cell, Photosensitivity Disorders, Postoperative Complications, Skin Diseases, Skin Neoplasms, Squamous Cell Carcinoma of Head and Neck, Vitiligo	Carcinoma, Squamous Cell (ClinicalTrials, ClinicalTrials, ClinicalTrials)

The ***Disease trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for the analyzed pathology.

Drugs approved in clinical trials

Table 16. 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 score	Disease activity score	Disease trial phase
Erlotinib	RPS6KA3, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, ILK, EGFR, SYK, EIF2AK2, BIRC5, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	99	6	Phase 3: Carcinoma, Squamous Cell, Brain Neoplasms, Carcinoma, Carcinoma, Adenosquamous, Carcinoma, Hepatocellular, Carcinoma, Large Cell, Carcinoma, Non-Small-Cell Lung, Head and Neck Neoplasms, Lung Neoplasms, Neoplasm Metastasis, Neoplasms, Thoracic Neoplasms
Tegafur	ITGA6, VEGFA, ITGB5, EGFR, ITGB1, PTK2, ITGB4, ITGA3	95	2	Phase 2: Carcinoma, Squamous Cell, Adenocarcinoma, Ascites, Bile Duct Neoplasms, Biliary Tract Neoplasms, Breast Neoplasms, Carcinoma, Carcinoma, Hepatocellular, Carcinoma, Non-Small-Cell Lung, Colorectal Neoplasms, Esophageal Neoplasms, Esophageal Squamous Cell Carcinoma, Gastrointestinal Neoplasms, Head and Neck Neoplasms, Intestinal Neoplasms, Nasopharyngeal Carcinoma, Nasopharyngeal Neoplasms, Neoplasm Metastasis, Neoplasms, Neoplasms, Unknown Primary, Pancreatic Neoplasms, Rectal Neoplasms, Stomach Neoplasms
Gefitinib	RPS6KA3, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, EGFR, SYK, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	94	3	Phase 2: Carcinoma, Squamous Cell, Adenocarcinoma, Adenocarcinoma of Lung, Brain Neoplasms, Breast Neoplasms, Carcinoid Tumor, Carcinoma, Carcinoma, Islet Cell, Carcinoma, Non-Small-Cell Lung, Carcinoma, Renal Cell, Carcinoma, Transitional Cell, Fallopian Tube Neoplasms, Gastrinoma, Gastrointestinal Neoplasms, Glioblastoma, Glucagonoma, Head and Neck Neoplasms, Insulinoma, Intestinal Neoplasms, Lung Diseases, Lung Neoplasms, Malignant Carcinoid Syndrome, Mesothelioma, Mesothelioma, Malignant, Nasopharyngeal Carcinoma, Nasopharyngeal Neoplasms, Neoplasm Metastasis, Neoplasms, Neoplasms, Squamous Cell, Neuroblastoma, Neuroendocrine Tumors, Ovarian Neoplasms, Peritoneal Neoplasms, Recurrence, Respiratory Tract Diseases, Respiratory Tract Neoplasms, Sarcoma, Sarcoma, Synovial, Somatostatinoma, Squamous Cell Carcinoma of Head and Neck, Stomach Neoplasms, Thoracic Neoplasms, Urinary Bladder Neoplasms, Vipoma
Lapatinib	RPS6KA3, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, EGFR, SYK, EIF2AK2, BIRC5, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	93	2	Phase 2: Carcinoma, Squamous Cell, Adenocarcinoma, Brain Neoplasms, Breast Diseases, Breast Neoplasms, Carcinoma, Carcinoma, Ductal, Carcinoma, Ductal, Breast, Carcinoma, Non-Small-Cell Lung, Carcinoma, Small Cell, Carcinoma, Transitional Cell, Fibroma, Glioblastoma, Glioma, Gliosarcoma, Head and Neck Neoplasms, Liver Neoplasms, Neoplasm Metastasis, Neoplasms, Neurilemmoma, Neuroblastoma, Neurofibroma, Neurofibromatoses, Neurofibromatosis 1, Neurofibromatosis 2, Neuroma, Neuroma, Acoustic, Sarcoma, Small Cell Lung Carcinoma, Squamous Cell Carcinoma of Head and Neck, Thymoma, Urinary Bladder Neoplasms

Crizotinib	MET, SRC, NTRK2, EPHB4, CSF1R, IGF1R	93	3	Phase 2: Carcinoma, Squamous Cell, Adenocarcinoma, Brain Abscess, Breast Neoplasms, Carcinoma, Carcinoma, Hepatocellular, Carcinoma, Non-Small-Cell Lung, Carcinoma, Ovarian Epithelial, Carcinoma, Renal Cell, Carcinoma, Transitional Cell, Colonic Neoplasms, Colorectal Neoplasms, Endometrial Neoplasms, Esophageal Neoplasms, Fibroma, Glioma, Kidney Neoplasms, Lung Neoplasms, Lymphoma, Lymphoma, Non-Hodgkin, Melanoma, Multiple Myeloma, Neoplasms, Neoplasms, Plasma Cell, Neurilemmoma, Neuroblastoma, Neurofibroma, Neurofibromatoses, Neurofibromatosis 1, Neurofibromatosis 2, Neuroma, Neuroma, Acoustic, Ovarian Neoplasms, Pancreatic Neoplasms, Prostatic Neoplasms, Rectal Neoplasms, Recurrence, Skin Neoplasms, Stomach Neoplasms, Thyroid Diseases, Thyroid Neoplasms, Ureteral Neoplasms, Urethral Neoplasms, Urinary Bladder Neoplasms, Uterine Neoplasms

The ***Disease trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for the analyzed pathology.

Repurposing drugs

Table 17. 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 score	Maximum trial phase
seliciclib	RPS6KA3, ROCK2, MET, NEK7, PAK2, GSK3B, MAP3K11, CDK4, PRKAA1, EGFR, SYK, SGK1, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	89	Phase 2: Cystic Fibrosis, Cysts, Fibrosis
Tofacitinib	RPS6KA3, ROCK2, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, EGFR, SYK, SGK1, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	89	Phase 4: Alopecia, Alopecia Areata, Aortic Arch Syndromes, Arteritis, Arthritis, Arthritis, Psoriatic, Arthritis, Rheumatoid, COVID-19, Colitis, Colitis, Ulcerative, Embolism, Granuloma, Granulomatosis with Polyangiitis, Necrosis, ST Elevation Myocardial Infarction, Spondylarthritis, Spondylitis, Systemic Vasculitis, Takayasu Arteritis, Thromboembolism, Ulcer, Vasculitis
1-(5-Tert-Butyl-2-P-Tolyl-2h-Pyrazol-3-Yl)-3-[4-(2-Morpholin-4-Yl-Ethoxy)-Naphthalen-1-Yl]-Urea	RPS6KA3, ROCK2, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, EGFR, SYK, SGK1, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	89	Phase 2: Arthritis, Arthritis, Rheumatoid, Psoriasis
pi-103	RPS6KA3, ROCK2, MET, NEK7, PAK2, GSK3B, MAP3K11, PRKAA1, EGFR, SYK, SGK1, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	89	N/A
Flavopiridol	RPS6KA3, CDK6, MET, NEK7, PAK2, GSK3B, MAP3K11, CDK4, PRKAA1, EGFR, SYK, EIF2AK2, IGF1R, JAK1, TGFBR1, SRC, CSNK2A2, PRKD3, NTRK2, CSNK1G2, EPHB4, YES1, BRAF, STK11, STK4, PTK2, PIK3CA, LYN, CSNK2A1, PRKACB, CSF1R	89	Phase 2: Embolism, Head and Neck Neoplasms, Lymphoma, Lymphoma, B-Cell, Lymphoma, Large B-Cell, Diffuse, Lymphoma, Mantle-Cell, Lymphoma, Non-Hodgkin, Neoplasms, Sarcoma, Thromboembolism

The ***Maximum trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for any pathology.

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

Table 18. 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 score	Target activity score
{(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE	CCND1, CDK6, CCND3, CCNB1, CCNA2, CCNB2, CDK4	100	3.82
3-Bromo-7-Nitroindazole	RPS6KA3, CCND1, CDK6, CCND3, CCNB1, GSK3B, CCNA2, CCNB2, CDK4	100	2.53
O6-CYCLOHEXYLMETHOXY-2-(4'-SULPHAMOYLANILINO) PURINE	CCND1, CDK6, CCND3, CCNB1, CCNA2, CDK4, CCNB2	100	2.46
N~6~-cyclohexyl-N~2~-(4-morpholin-4-ylphenyl)-9H-purine-2,6-diamine	CDK6, SRC, CSNK2A2, CCND3, CCNB1, YES1, CCNB2, CDK4, CCND1, SYK, LYN, CCNA2, CSNK2A1, JAK1	100	2.38
2-ANILINO-6-CYCLOHEXYLMETHOXYPURINE	CCND1, CDK6, CCND3, CCNB1, CCNA2, CDK4, CCNB2	99	2.32

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: Erlotinib, seliciclib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE. These drugs were selected for acting on the following targets: NTRK2 and CCND3, 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.

Supplementary drug info

In addition to the approved and repurposed drugs proposed by Genome Enhancer, below the ***Supplementary drug info*** table is given, which contains an extended list of drugs used for treatment of neoplasms. Those drugs which were predicted by Genome Enhancer as prospective treatment candidates for the studied case (both approved and repurposed) have a respective ***Predicted Drug Score*** assigned to them. This value on a scale from 1 to 100 reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. The ***Predicted Drug Score*** column contains the "-" (Not Identified) value in case the drug targets of the respective treatment were not found in the molecular mechanism of the studied pathology.

Table 19. Supplementary drug info: extended list of drugs used for treatment of neoplasms with respective drug scores predicted for the studied pathology.

Drug	Disease	Predicted Drug Score
Abarelix	Prostatic Neoplasms	-
Abemaciclib	Breast Neoplasms	79
Abiraterone	Prostatic Neoplasms, Castration-Resistant	-
Abiraterone acetate	Prostatic Neoplasms, Castration-Resistant	-
Acalabrutinib	Lymphoma, Mantle-Cell	-
Acitretin	Psoriasis	-
Ado-trastuzumab emtansine	Breast Neoplasms|Neoplasms	42
Afatinib	Carcinoma, Non-Small-Cell Lung	52
Aflibercept	Colorectal Neoplasms|Diabetic Retinopathy|Edema|Vascular Diseases|Wet Macular Degeneration	26

Alectinib	Carcinoma, Non-Small-Cell Lung	-
Alemtuzumab	Brain Abscess|Leukemia, Lymphocytic, Chronic, B-Cell|Multiple Sclerosis|Multiple Sclerosis, Relapsing-Remitting|Sclerosis	-
Alitretinoin	Sarcoma, Kaposi	-
Alpelisib	Breast Neoplasms	78
Altretamine	Ovarian Neoplasms	-
Aminolevulinic acid	Keratosis|Keratosis, Actinic	-
Anagrelide	Thrombocythemia, Essential|Thrombocytosis	-
Anastrozole	Breast Neoplasms|Hypersensitivity|Obesity|Obesity, Morbid|Recurrence|Weight Loss	-
Apalutamide	Prostatic Neoplasms, Castration-Resistant	-
Aprepitant	Nausea|Neoplasms|Postoperative Nausea and Vomiting	-
Arsenic trioxide	Leukemia, Promyelocytic, Acute	60
Atezolizumab	Carcinoma, Non-Small-Cell Lung|Carcinoma, Transitional Cell|Triple Negative Breast Neoplasms	-
Avelumab	Carcinoma, Merkel Cell|Carcinoma, Renal Cell|Carcinoma, Transitional Cell	-
Axitinib	Carcinoma, Renal Cell	-
Azacitidine	Anemia, Refractory|Anemia, Refractory, with Excess of Blasts|Leukemia, Myelomonocytic, Chronic|Myelodysplastic Syndromes|Preleukemia|Syndrome	9
Belinostat	Lymphoma, T-Cell, Peripheral	22
Bendamustine	Leukemia, Lymphocytic, Chronic, B-Cell|Leukemia, Lymphoid	-
Bevacizumab	Breast Neoplasms|Colonic Neoplasms|Colorectal Neoplasms|Corneal Neovascularization|Diabetic Retinopathy|Dilatation, Pathologic|Edema|Epistaxis|Glaucoma|Hemorrhage|Macular Degeneration|Macular Edema|Neoplasm Metastasis|Neoplasms|Neovascularization, Pathologic|Optic Nerve Diseases|Pterygium|Rectal Neoplasms|Retinal Detachment|Retinal Diseases|Retinal Vein Occlusion|Telangiectasia, Hereditary Hemorrhagic|Telangiectasis|Vitreous Hemorrhage	20
Bexarotene	Lymphoma, T-Cell|Lymphoma, T-Cell, Cutaneous	-
Bicalutamide	Prostatic Neoplasms	-
Binimetinib	Melanoma	-
Blinatumomab	Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	-
Bortezomib	Brain Abscess|Glomerulonephritis|Glomerulonephritis, IGA|Kidney Diseases|Multiple Myeloma|Neoplasms, Plasma Cell|Nephritis|Renal Insufficiency	9
Bosutinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	68
Brentuximab vedotin	Hodgkin Disease|Lymphoma|Lymphoma, Large-Cell, Anaplastic|Lymphoma, T-Cell, Peripheral	-
Brigatinib	Carcinoma, Non-Small-Cell Lung	68
Buserelin	Prostatic Neoplasms	-
Cabazitaxel	Prostatic Neoplasms, Castration-Resistant	28
Cabergoline	Drug-Related Side Effects and Adverse Reactions|Pituitary Neoplasms	-
Cabozantinib	Thyroid Neoplasms	79
Capecitabine	Breast Neoplasms|Colonic Neoplasms|Colorectal Neoplasms	-
Carboplatin	Carcinoma, Non-Small-Cell Lung|Lung Neoplasms|Neoplasms|Neuroendocrine Tumors|Ovarian Neoplasms|Retinoblastoma	-
Carfilzomib	Multiple Myeloma	20
Carmustine	Astrocytoma|Glioblastoma|Hodgkin Disease|Medulloblastoma|Multiple Myeloma|Neoplasms	-
Ceritinib	Carcinoma, Non-Small-Cell Lung	62
Cetuximab	Colorectal Neoplasms	41
Cinacalcet	Anemia|Calcinosis|Cardiovascular Diseases|Hyperparathyroidism|Hyperparathyroidism, Secondary|Kidney Diseases|Kidney Failure, Chronic|Neoplasm Metastasis|Neoplasms|Parathyroid Neoplasms|Renal Insufficiency|Vascular Calcification|Vascular Diseases|Vision Disorders	-

Cisplatin	Carcinoma, Squamous Cell|Neoplasms|Uterine Cervical Neoplasms|Carcinoma, Non-Small-Cell Lung|Esophageal Neoplasms|Carcinoma	-
Cladribine	Leukemia, Hairy Cell	-
Clofarabine	Precursor Cell Lymphoblastic Leukemia-Lymphoma	3
Cobimetinib	Melanoma	-
Copanlisib	Lymphoma, Follicular	57
Crizotinib	Carcinoma, Non-Small-Cell Lung	93
Cyproterone acetate	Prostatic Neoplasms	-
Dabrafenib	Melanoma	13
Dacomitinib	Carcinoma, Non-Small-Cell Lung	71
Daratumumab	Multiple Myeloma	-
Dasatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive|Leukemia, Myeloid, Chronic-Phase|Precursor Cell Lymphoblastic Leukemia-Lymphoma	85
Decitabine	Anemia, Refractory|Anemia, Refractory, with Excess of Blasts|Leukemia, Myelomonocytic, Chronic|Myelodysplastic Syndromes	-
Degarelix	Cardiovascular Diseases|Prostatic Neoplasms|Vascular Diseases	-
Denosumab	Arthritis, Rheumatoid|Bone Diseases|Bone Diseases, Metabolic|Breast Neoplasms|Hyperparathyroidism|Hyperparathyroidism, Primary|Metabolic Diseases|Neoplasm Metastasis|Neoplasms|Osteoporosis|Osteoporosis, Postmenopausal|Prostatic Neoplasms	-
Dexrazoxane	Breast Neoplasms|Cardiomyopathies	-
Dienogest	Menorrhagia	-
Dinutuximab	Neuroblastoma	-
Docetaxel	Breast Neoplasms|Carcinoma, Non-Small-Cell Lung|Prostatic Neoplasms|Squamous Cell Carcinoma of Head and Neck|Stomach Neoplasms	-
Doxorubicin	Neoplasms|Multiple Myeloma|Carcinoma, Ovarian Epithelial|Ovarian Neoplasms|Leukemia, Lymphoid|Breast Neoplasms|Lymphoma, Follicular|Thyroid Neoplasms|Triple Negative Breast Neoplasms|Glioma	76
Durvalumab	Carcinoma, Non-Small-Cell Lung|Carcinoma, Transitional Cell	-
Dutasteride	Alcoholism|Hyperplasia|Hypertrophy|Neoplasms|Prostatic Hyperplasia	-
Duvelisib	Leukemia, Lymphocytic, Chronic, B-Cell|Lymphoma, Follicular	-
Elotuzumab	Multiple Myeloma	-
Enasidenib	Leukemia, Myeloid, Acute	-
Encorafenib	Colorectal Neoplasms|Melanoma	5
Enfortumab vedotin	Carcinoma, Transitional Cell|Neoplasms	-
Entrectinib	Carcinoma, Non-Small-Cell Lung	71
Enzalutamide	Prostatic Neoplasms|Prostatic Neoplasms, Castration-Resistant	-
Epirubicin	Breast Neoplasms	39
Erdafitinib	Urinary Bladder Neoplasms	53
Eribulin	Breast Neoplasms|Drug-Related Side Effects and Adverse Reactions|Neoplasms	-
Erlotinib	Carcinoma, Non-Small-Cell Lung|Neoplasms|Pancreatic Neoplasms	99
Erlotinib hydrochloride	Carcinoma, Non-Small-Cell Lung|Gastrointestinal Stromal Tumors	-
Estramustine	Prostatic Neoplasms	-
Ethinyl Estradiol	Acne Vulgaris|Neoplasms	10
Everolimus	Angiomyolipoma|Arthrogryposis|Astrocytoma|Breast Neoplasms|Carcinoma, Renal Cell|Cysts|Idiopathic Pulmonary Fibrosis|Kidney Diseases, Cystic|Kidney Failure, Chronic|Lipoma|Neuroendocrine Tumors|Primary Graft Dysfunction|Sclerosis|Tuberous Sclerosis	52
Exemestane	Breast Neoplasms	-
Fedratinib	Primary Myelofibrosis	-
Finasteride	Hyperplasia|Neoplasms|Prostatic Hyperplasia	-
Flavopiridol	Leukemia, Lymphocytic, Chronic, B-Cell	89
Fluorouracil	Skin Neoplasms|Neoplasms, Basal Cell|Neoplasms, Second Primary|Neoplasms, Squamous Cell|Neoplasms|Colorectal Neoplasms|Pancreatic Neoplasms	52
Fluoxymesterone	Breast Neoplasms|Hypogonadism|Puberty, Delayed	-

Flutamide	Premenstrual Dysphoric Disorder|Premenstrual Syndrome|Prostatic Neoplasms	50
Fulvestrant	Breast Neoplasms	-
Gefitinib	Carcinoma, Non-Small-Cell Lung	94
Gemcitabine	Breast Neoplasms|Carcinoma, Non-Small-Cell Lung|Ovarian Neoplasms|Pancreatic Neoplasms	-
Gemtuzumab ozogamicin	Leukemia, Myeloid, Acute	-
Gilteritinib	Leukemia, Myeloid, Acute	64
Glasdegib	Leukemia, Myeloid, Acute	-
Goserelin	Atrophy|Breast Neoplasms|Bulbo-Spinal Atrophy, X-Linked|Endometriosis|Muscular Atrophy|Myoma|Prostatic Neoplasms	-
Histrelin	Puberty, Precocious	-
Homoharringtonine	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	65
Ibritumomab	Lymphoma, B-Cell|Lymphoma, Follicular	-
Ibrutinib	Graft vs Host Disease|Leukemia, Lymphocytic, Chronic, B-Cell|Lymphoma, B-Cell, Marginal Zone|Lymphoma, Mantle-Cell|Waldenstrom Macroglobulinemia	15
Idarubicin	Leukemia, Myeloid, Acute	-
Idelalisib	Leukemia, Lymphocytic, Chronic, B-Cell|Lymphoma, Follicular	-
Ifosfamide	Neoplasms	-
Imatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive|Mastocytosis, Systemic|Neoplasms	89
Inotuzumab ozogamicin	Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	-
Ipilimumab	Carcinoma, Renal Cell|Melanoma	-
Irinotecan	Colorectal Neoplasms	59
Ivosidenib	Leukemia, Myeloid, Acute	-
Ixabepilone	Breast Neoplasms	-
Ixazomib	Multiple Myeloma	-
Lapatinib	Breast Neoplasms	93
Larotrectinib	Neoplasm Metastasis	79
Lenalidomide	Brain Abscess|Lupus Erythematosus, Cutaneous|Myelodysplastic Syndromes|Neoplasms, Plasma Cell	-
Lenvatinib	Carcinoma, Hepatocellular|Carcinoma, Renal Cell|Thyroid Neoplasms	-
Letrozole	Breast Neoplasms|Cysts|Fibroma|Myofibroma|Myoma|Ovarian Cysts|Syndrome	-
Leuprolide	Hot Flashes|Ovarian Hyperstimulation Syndrome|Prostatic Neoplasms|Puberty, Precocious	-
Levamisole	Ascariasis|Colonic Neoplasms|Helminthiasis	-
Levonorgestrel	Epilepsy|Hyperplasia|Menorrhagia	-
Lomustine	Brain Neoplasms|Hodgkin Disease	-
Lonafarnib	Breast Neoplasms|Carcinoma, Non-Small-Cell Lung|Central Nervous System Neoplasms|Colorectal Neoplasms|Gliosarcoma|Head and Neck Neoplasms|Leukemia, Myelomonocytic, Chronic| Liver Neoplasms|Lymphoma|Myelodysplastic Syndromes|Ovarian Neoplasms|Urethral Neoplasms|Urinary Bladder Neoplasms	62
Lorlatinib	Carcinoma, Non-Small-Cell Lung	82
Masoprocol	Keratosis, Actinic	-
Medroxyprogesterone Acetate	Depression|Depression, Postpartum|Depressive Disorder|Metrorrhagia|Neoplasms|Uterine Hemorrhage	-
Megestrol acetate	Acquired Immunodeficiency Syndrome|Bites and Stings|Breast Neoplasms|Pain|Wasting Syndrome	-
Methotrexate	Neoplasms|Breast Neoplasms|Head and Neck Neoplasms|Ovarian Neoplasms|Lymphoma, T-Cell, Peripheral|Brain Neoplasms|Colorectal Neoplasms|Neuroblastoma|Carcinoma, Squamous Cell	39
Methyltestosterone	Breast Neoplasms|Hypogonadism|Puberty, Delayed	-
Midostaurin	Leukemia, Mast-Cell|Leukemia, Myeloid, Acute|Mastocytosis, Systemic	85
Mitotane	Adrenocortical Carcinoma	-
Mitoxantrone	Autoimmune Diseases|Autoimmune Diseases of the Nervous System|Demyelinating Autoimmune Diseases, CNS|Immune System	-

	Diseases|Leukemia, Myeloid, Acute|Multiple Sclerosis|Myelitis|Myelitis, Transverse|Nervous System Diseases|Neuromyelitis Optica|Prostatic Neoplasms, Castration-Resistant	
Mogamulizumab	Mycosis Fungoides|Neoplasms|Sezary Syndrome	-
Moxetumomab pasudotox	Leukemia, Hairy Cell|Neoplasms	-
Necitumumab	Carcinoma, Non-Small-Cell Lung|Neoplasms	-
Nelarabine	Precursor T-Cell Lymphoblastic Leukemia-Lymphoma	-
Neratinib	Breast Neoplasms	61
Nilotinib	Blast Crisis|Leukemia, Myelogenous, Chronic, BCR-ABL Positive|Leukemia, Myeloid, Chronic-Phase	13
Nilutamide	Prostatic Neoplasms	-
Nintedanib	Fibrosis|Idiopathic Pulmonary Fibrosis	47
Niraparib	Carcinoma, Ovarian Epithelial|Fallopian Tube Neoplasms|Peritoneal Neoplasms	65
Nivolumab	Carcinoma, Non-Small-Cell Lung|Kidney Neoplasms|Neoplasms|Lung Neoplasms|Melanoma	-
Obinutuzumab	Leukemia, Lymphocytic, Chronic, B-Cell	-
Octreotide	Acromegaly|Adenoma|Ascites|Carcinoid Tumor|Fistula|Pancreatic Fistula|Pituitary Diseases|Renal Insufficiency|Vipoma	-
Ofatumumab	Leukemia, Lymphocytic, Chronic, B-Cell	-
Olaparib	Breast Neoplasms|Carcinoma, Ovarian Epithelial|Fallopian Tube Neoplasms|Ovarian Neoplasms|Pancreatic Neoplasms|Peritoneal Neoplasms|Prostatic Neoplasms, Castration-Resistant	53
Olaratumab	Sarcoma	-
Osimertinib	Carcinoma, Non-Small-Cell Lung	27
Oxaliplatin	Colonic Neoplasms|Colorectal Neoplasms|Neoplasms|Rectal Neoplasms	-
Paclitaxel	Acute Coronary Syndrome|Angina Pectoris|Arteriosclerosis|Breast Neoplasms|Carcinoma, Non-Small-Cell Lung|Cardiovascular Diseases|Coronary Artery Disease|Coronary Disease|Coronary Stenosis|Heart Diseases|Myocardial Ischemia|Ovarian Neoplasms|Vascular Diseases	44
Palbociclib	Breast Neoplasms	76
Panitumumab	Colorectal Neoplasms	75
Panobinostat	Multiple Myeloma	-
Pazopanib	Carcinoma|Carcinoma, Renal Cell|Sarcoma	87
Pembrolizumab	Carcinoma, Hepatocellular|Carcinoma, Merkel Cell|Carcinoma, Non-Small-Cell Lung|Carcinoma, Renal Cell|Carcinoma, Transitional Cell|Hodgkin Disease|Melanoma|Neoplasms|Stomach Neoplasms	-
Pemetrexed	Carcinoma, Non-Small-Cell Lung|Mesothelioma	-
Pentostatin	Leukemia, Hairy Cell	30
Pertuzumab	Breast Neoplasms	-
Pomalidomide	Multiple Myeloma	-
Ponatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive|Precursor Cell Lymphoblastic Leukemia-Lymphoma	81
Pralatrexate	Lymphoma, T-Cell, Peripheral	-
Radium Ra 223 Dichloride	Prostatic Neoplasms, Castration-Resistant	-
Ramucirumab	Stomach Neoplasms	-
Rasburicase	Hyperuricemia|Leukemia|Lymphoma|Neoplasms|Syndrome|Tumor Lysis Syndrome	-
Regorafenib	Colorectal Neoplasms	34
Relugolix	Prostatic Neoplasms	-
Ribociclib	Breast Neoplasms	80
Rituximab	Arthritis|Arthritis, Rheumatoid|Granulomatosis with Polyangiitis|Leukemia|Leukemia, Lymphoid|Lymphoma|Lymphoma, B-Cell|Lymphoma, Follicular|Lymphoma, Non-Hodgkin|Myelitis|Neuromyelitis Optica|Purpura|Purpura, Thrombocytopenic|Purpura, Thrombocytopenic, Idiopathic|Thrombocytopenia	-
Romidepsin	Lymphoma, T-Cell, Cutaneous	-

Rucaparib	Carcinoma, Ovarian Epithelial|Fallopian Tube Neoplasms|Peritoneal Neoplasms|Prostatic Neoplasms, Castration-Resistant	40
Ruxolitinib	Graft vs Host Disease|Polycythemia|Polycythemia Vera|Primary Myelofibrosis|Thrombocytosis	3
Selinexor	Multiple Myeloma	-
Selumetinib	Neurofibromatosis 1	-
Siltuximab	Giant Lymph Node Hyperplasia	-
Sirolimus	Angiomyolipoma|Constriction, Pathologic|Coronary Restenosis|Eye Diseases|Immune System Diseases|Kidney Failure, Chronic|Lipoma|Tuberous Sclerosis	74
Sonidegib	Carcinoma, Basal Cell	-
Sorafenib	Carcinoma, Hepatocellular|Carcinoma, Renal Cell|Thyroid Neoplasms	88
Sunitinib	Adenoma|Carcinoma, Renal Cell|Digestive System Neoplasms|Gastrointestinal Neoplasms|Gastrointestinal Stromal Tumors|Intestinal Neoplasms	85
Talazoparib	Breast Neoplasms	39
Tamoxifen	Breast Diseases|Cystic Fibrosis|Cysts|Fibroadenoma|Fibrocystic Breast Disease|Hemorrhage|Menorrhagia|Menstruation Disturbances|Metrorrhagia|Neoplasms	54
Tamsulosin	Calculi|Coronary Artery Disease|Heart Diseases|Hernia|Hernia, Inguinal|Inflammation|Ischemia|Lithiasis|Lower Urinary Tract Symptoms|Myocardial Ischemia|Prostatic Hyperplasia|Ureteral Calculi|Urinary Calculi|Urolithiasis|Urologic Diseases	-
Temozolomide	Astrocytoma|Nervous System Neoplasms	-
Temsirolimus	Carcinoma, Renal Cell	67
Teniposide	Precursor Cell Lymphoblastic Leukemia-Lymphoma	9
Thalidomide	Brain Abscess|Immune System Diseases|Multiple Myeloma|Neoplasms, Plasma Cell	-
Tivozanib	Carcinoma, Renal Cell	-
Tocilizumab	Arthritis|Arthritis, Juvenile|Arthritis, Rheumatoid|Behavior|Cytokine Release Syndrome|Giant Cell Arteritis|Neurobehavioral Manifestations|Oral Manifestations|Psychotic Disorders|Schizophrenia|Tic Disorders	-
Topotecan	Small Cell Lung Carcinoma	-
Toremifene	Breast Neoplasms	-
Trabectedin	Leiomyosarcoma|Liposarcoma	-
Trametinib	Carcinoma, Non-Small-Cell Lung|Melanoma	31
Trastuzumab	Breast Neoplasms|Neoplasms	34
Tretinoin	Lentigo	86
Triptorelin	Fatty Liver|Hypogonadism|Infertility, Female|Prostatic Neoplasms	20
Tucatinib	Breast Neoplasms	-
Valrubicin	Urinary Bladder Neoplasms	-
Vandetanib	Thyroid Neoplasms	93
Vemurafenib	Melanoma	6
Venetoclax	Leukemia, Lymphocytic, Chronic, B-Cell|Leukemia, Myeloid, Acute	-
Vinblastine	Glioma	-
Vincristine	Precursor Cell Lymphoblastic Leukemia-Lymphoma	-
Vinorelbine	Carcinoma, Non-Small-Cell Lung	-
Vismodegib	Carcinoma, Basal Cell	-
Vorinostat	Lymphoma, T-Cell, Cutaneous	24
Zoledronate	Arthritis|Bone Marrow Diseases|Brain Abscess|Chronic Kidney Disease-Mineral and Bone Disorder|Chronic Periodontitis|HIV Infections|Hypersensitivity|Infections|Kidney Diseases|Metabolic Diseases|Multiple Myeloma|Neoplasms|Neoplasms, Plasma Cell|Neoplasms, Second Primary|Osteitis|Osteoarthritis|Periodontitis|Pleural Effusion, Malignant|Prostatic Neoplasms|Renal Insufficiency, Chronic|Thalassemia|Wounds and Injuries	-

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 *Squamous Cell Carcinoma*. 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:

Erlotinib, seliciclib and {(2Z)-4-AMINO-2-[(4-METHOXYPHENYL)IMINO]-2,3-DIHYDRO-1,3-THIAZOL-5-YL}(4-METHOXYPHENYL)METHANONE

These drugs were selected for acting on the following targets: NTRK2 and CCND3, 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:

ornithine decarboxylase and trkB

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: 1-(5-OXO-2,3,5,9B-TETRAHYDRO-1H-PYRROLO[2,1-A]ISOINDOL-9-YL)-3-(5-PYRROLIDIN-2-YL-1H-PYRAZOL-3-YL)-UREA, Erlotinib, seliciclib, Methyl aminolevulinate and Eflornithine. 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.

- ornithine decarboxylase
- trkB

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 2023.2 (geneXplain GmbH, Wolfenbüttel, Germany) (https://genexplain.com/transfac).
The master regulator search uses the TRANSPATH® database (BIOBASE), release 2023.2 (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 2023.2 (https://genexplain.com/humanpsd).

The Ensembl database release Human104.38 (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 the sum of the following ranks:

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

"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" ($D\text{-}score_{PSD}$):

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

where *D* is the set of selected diseases, and if *D* is empty set, $D\text{-}score_{PSD}=0$. *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.
The clinical validity score reflects the number of the highest clinical trials phase (from 1 to 4) on which the drug was ever tested for any pathology.

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 Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).
4. Supplementary table 4 - Detailed report. Composite modules and master regulators (down-regulated genes in Experiment: Squamous Cell Carcinoma vs. Control: Non-tumour tissue).
5. Supplementary table 5 - Detailed report. Pharmaceutical compounds and drug targets.

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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

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