养阴通脑颗粒中关键成分对脑缺血再灌注的影响

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LiChuang Huang

@ 立效研究院

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1 摘要

1.1 需求

- 养阴通脑颗粒中治疗脑缺血再灌注的关键成分及相应信号通路（信号通路需要创新性的），1-3 条
- 同时重点分析水蛭素对应的治疗脑缺血再灌注的信号通路

养阴通脑颗粒：地黄 15g、黄芪 15g、葛根 18g、石斛 15g、水蛭 3g、川芎 9g

1.2 结果

1.2.1 整体复方

- 常规网络药理学，见 Fig. 3, 富集结果见 Fig. 7
- 额外对 CIR 的 GEO 数据差异分析，富集结果 Fig. 9
- 综合以上富集，发现 MARK 通路 (Fig. 10) 可能是治疗的关键通路之一，其靶向成分见 Tab. 10

1.2.2 权衡 Hirudin 的作用

HERBs 数据库 (其他数据库也是如此) 包含的 Hirudin 靶点较少。这里，额外从 GeneCards 获取了 Hirudin 的靶点 (Tab. 12)。

为了缩小可选通路范围，这里尝试将以下的富集结果取共同的交集 (已在上述部分完成)：

- 复方靶向 CIR (靶点来源见 Fig. 2) 的通路 (富集见 Fig. 7)
- GEO 数据集 (GSE163614) CIR DEGs 的富集结果的通路 (富集见 Fig. 9)
- 获取了更多靶点信息 (因为 HERBS 数据库或其他数据库包含的靶点信息太少，不利于分析) 的 Hirudin 靶向 CIR (GEO DEGs) 的基因的富集分析 (Fig. 13)

得到 (去除了名称包含其他疾病的通路)：Tab. 14

- HIF-1 signaling pathway
- Apelin signaling pathway

更多信息见 6.3.1 和 6.3.2

2 前言

3 材料和方法

3.1 材料

All used GEO expression data and their design:

- **GSE163614**: Examination of MCAO/R and Sham rat brain samples（n=3）

3.2 方法

Mainly used method:

- The `BindingDB` database was used for discovering association between Ligands and Receptors[1].
- The `biomart` was used for mapping genes between organism (e.g., mgi_symbol to hgnc_symbol)[2].
- R package `ClusterProfiler` used for gene enrichment analysis[3].
- GEO https://www.ncbi.nlm.nih.gov/geo/ used for expression dataset aquisition.
- Databses of `DisGeNet`, `GeneCards`, `PharmGKB` used for collating disease related targets[4–6].
- Website `HERB` http://herb.ac.cn/ used for data source[7].
- R package `Limma` and `edgeR` used for differential expression analysis[8,9].
- R package `PubChemR` used for querying compounds information.
- R package `STEINGdb` used for PPI network construction[10,11].
- Web tool of `Super-PRED` used for drug-targets relationship prediction[12].
- The MCC score was calculated referring to algorithm of `CytoHubba`[11].
- R package `UniProt.ws` used for querying Gene or Protein information.
- R version 4.3.2 (2023-10-31); Other R packages (eg., `dplyr` and `ggplot2`) used for statistic analysis or data visualization.

4 分析结果

5 结论

6 附：分析流程

6.1 养阴通脑颗粒

6.1.1 成分

Table 1 (下方表格) 为表格 Herbs information 概览。

(对应文件为 `Figure+Table/Herbs-information.xlsx`)

> 注：表格共有 6 行 18 列，以下预览的表格可能省略部分数据；表格含有 6 个唯一 'Herb_'。

Table 1: Herbs information

Herb_	Herb_p…	Herb_c…	Herb_e…	Herb_l…	Proper…	Meridians	UsePart	Function	Indica…
HERB00…	CHUAN …	川芎	Chuanx…	Radix …	Warm; …	Liver;…	rhizome	1. To …	Cerebr…
HERB00…	DI HUANG	地黄	Radix …	NA	NA	NA	NA	NA	NA
HERB00…	GE GEN	葛根	root o…	Radix …	Cool; …	Spleen…	tuberoid	To rel…	Angina…
HERB00…	HUANG QI	黄芪	root o…	Radix …	Warm; …	Lung; …	root	To rei…	Common…
HERB00…	SHI HU	石斛	Dendro…	Herba …	Minor …	Stomac…	Dendro…	Treatm…	1. Den…

Herb_	Herb_p…	Herb_c…	Herb_e…	Herb_l…	Proper…	Meridians	UsePart	Function	Indica…
HERB00…	SHUI ZHI	水蛭	Bigflo…	Garden…	Mild; …	Liver	fruit	To cle…	Heat t…

Table 2 (下方表格) 为表格 Components of Herbs 概览。

(对应文件为 `Figure+Table/Components-of-Herbs.xlsx`)

> 注：表格共有 725 行 4 列，以下预览的表格可能省略部分数据；表格含有 696 个唯一 'Ingredient.name'。

Table 2: Components of Herbs

herb_id	Ingredient.id	Ingredient.name	Ingredient.alias
HERB002560	HBIN001244	13-hydroxy-9,11-o…	NA
HERB002560	HBIN002016	1,7-Dihydroxy-3,9…	1,7-dihydroxy-3,9…
HERB002560	HBIN003405	20-Hexadecanoylin…	20-hexadecanoylin…
HERB002560	HBIN003436	20(r)-21,24-cyclo…	20(r)-21,24-cyclo…
HERB002560	HBIN004319	2',4' -…	2', 4'-…
HERB002560	HBIN005731	2'-hydroxy-3	NA
HERB002560	HBIN005735	2'-hydroxy-3…	NA
HERB002560	HBIN005744	2-hydroxy-3-metho…	NA
HERB002560	HBIN006143	2-Nonyl acetate	ANW-21203; SCHEMB…
HERB002560	HBIN006743	(2S)-4-methoxy-7-…	(2S)-4-methoxy-7-…
HERB002560	HBIN007657	3,5-dimethoxystil…	78916-49-1; TR-03…
HERB002560	HBIN007848	3,9-di-O-methylni…	NA
HERB002560	HBIN008647	3-Hydroxy-2-picoline	BTB 09012; 3-Hydr…
HERB002560	HBIN008667	3'-hydroxy-4…	NA
HERB002560	HBIN008668	3'-Hydroxy-4…	3-(3-hydroxy-4-me…
…	…	…	…

Figure 1 (下方图) 为图 intersection of all compounds 概览。

(对应文件为 `Figure+Table/intersection-of-all-compounds.pdf`)

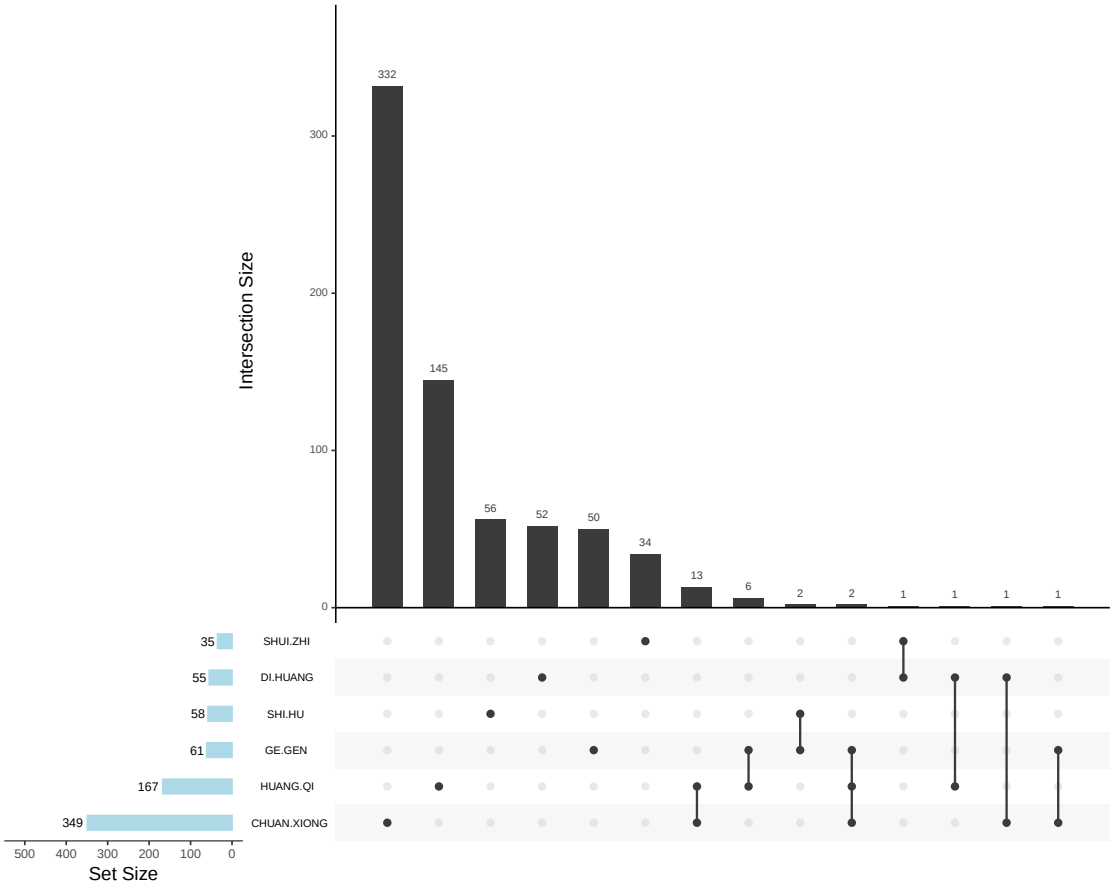

Figure 1: Intersection of all compounds

All_intersection :

(上述信息框内容已保存至 `Figure+Table/intersection-of-all-compounds-content`)

6.1.2 成分靶点

Table 3 (下方表格) 为表格 tables of Herbs compounds and targets 概览。

(对应文件为 `Figure+Table/tables-of-Herbs-compounds-and-targets.xlsx`)

注：表格共有 13356 行 9 列，以下预览的表格可能省略部分数据；表格含有 696 个唯一 'Ingredient.id'。

Table 3: Tables of Herbs compounds and targets

Ingred……1	Herb_p…	Ingred……3	Ingred……4	Target.id	Target…	Databa…	Paper.id	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	ATIC	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	FPGS	NA	NA	…

Ingred……1	Herb_p…	Ingred……3	Ingred……4	Target.id	Target…	Databa…	Paper.id	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	GART	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	MTHFD1	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	MTHFD2	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	ALDH1L1	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	MTHFD1L	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	MTFMT	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	ALDH1L2	NA	NA	…
HBIN00…	SHI HU	10,12-…	NA	HBTAR0…	MTHFD2L	NA	NA	…
HBIN00…	SHI HU	10 ,13…	NA	NA	NA	NA	NA	…
HBIN00…	CHUAN …	10-(be…	10-(-…	NA	NA	NA	NA	…
HBIN00…	CHUAN …	1,1-Di…	3658-9…	NA	NA	NA	NA	…
HBIN00…	CHUAN …	1,2,3,…	NA	NA	NA	NA	NA	…
HBIN00…	CHUAN …	1,3,8-…	1,3,8-…	HBTAR0…	ACHE	NA	NA	…
…	…	…	…	…	…	…	…	…

6.1.3 脑缺血再灌注 cerebral ischemia reperfusion (CIR) 靶点

Figure 2 (下方图) 为图 Overall targets number of datasets 概览。

(对应文件为 `Figure+Table/Overall-targets-number-of-datasets.pdf`)

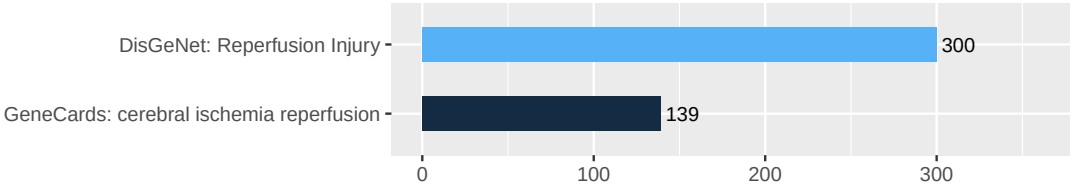

Figure 2: Overall targets number of datasets

The GeneCards data was obtained by querying :

cerebral ischemia reperfusion

Restrict (with quotes) :

TRUE

Filtering by Score: :

Score > 1

Table 4 (下方表格) 为表格 CIR GeneCards used data 概览。

(对应文件为 `Figure+Table/CIR-GeneCards-used-data.xlsx`)

> 注：表格共有 139 行 7 列，以下预览的表格可能省略部分数据；表格含有 139 个唯一 'Symbol'。

Table 4: CIR GeneCards used data

Symbol	Description	Category	UniProt_ID	GIFtS	GC_id	Score
BDNF-AS	BDNF Antis…	RNA Gene		28	GC11P027466	11.94
CERNA3	Competing …	RNA Gene		19	GC08P056101	6.64
MEG3	Maternally…	RNA Gene		34	GC14P115583	6.13
SNHG12	Small Nucl…	RNA Gene	Q9BXW3	29	GC01M030655	6.06
MIR211	MicroRNA 211	RNA Gene		28	GC15M031065	5.85
SNHG14	Small Nucl…	RNA Gene		24	GC15P147532	5.69
SOD2-OT1	SOD2 Overl…	RNA Gene		18	GC06M159772	5.41
H19	H19 Imprin…	RNA Gene		34	GC11M001995	4.64
GAS5	Growth Arr…	RNA Gene		30	GC01M173947	4.56
TUG1	Taurine Up…	Protein Co…	A0A6I8PU40	32	GC22P030969	4.15
MIR496	MicroRNA 496	RNA Gene		16	GC14P115621	4.07
BCL2	BCL2 Apopt…	Protein Co…	P10415	59	GC18M063123	3.7
MIR532	MicroRNA 532	RNA Gene		23	GC0XP056752	3.7
SCARNA5	Small Caja…	RNA Gene		23	GC02P233275	3.7
NFE2L2	NFE2 Like …	Protein Co…	Q16236	60	GC02M177227	3.64
…	…	…	…	…	…	…

6.1.4 网络药理-疾病

Figure 3 (下方图) 为图 Network pharmacology with disease 概览。

(对应文件为 `Figure+Table/Network-pharmacology-with-disease.pdf`)

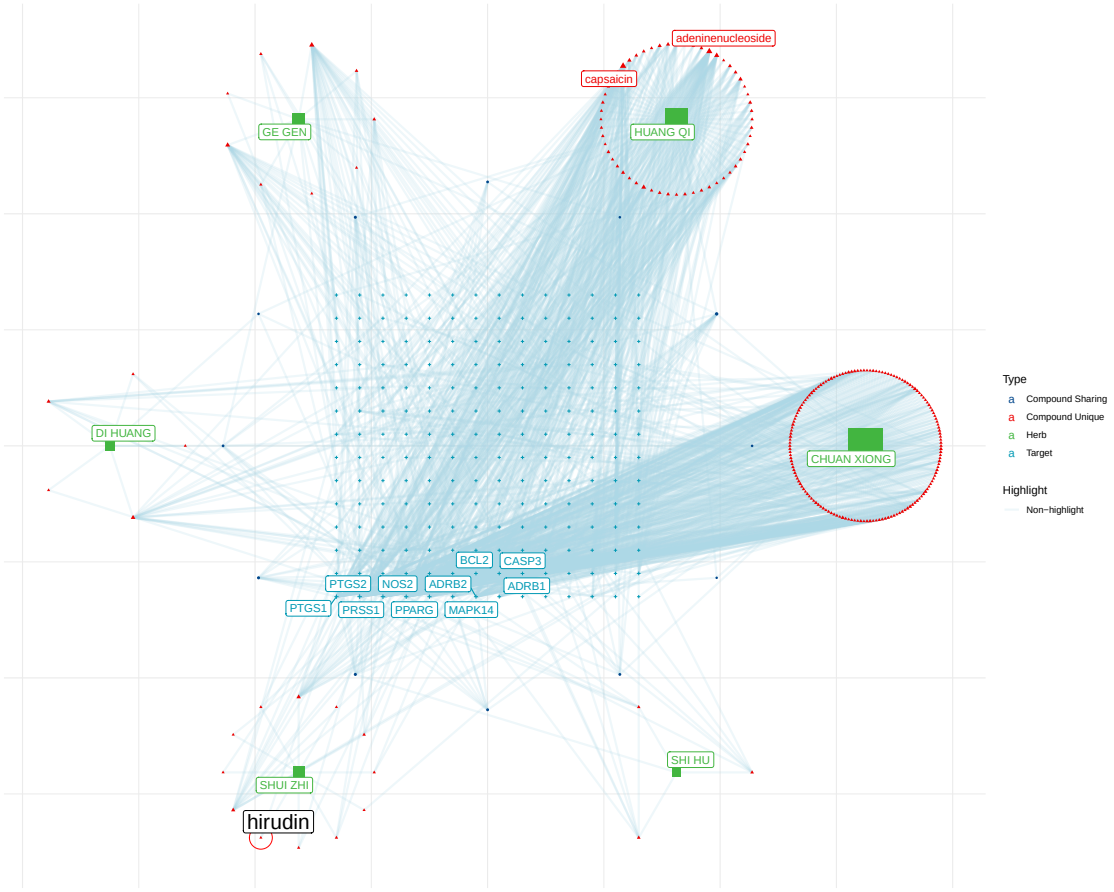

Figure 3: Network pharmacology with disease

Figure 4 (下方图) 为图 Targets intersect with targets of diseases 概览。

(对应文件为 `Figure+Table/Targets-intersect-with-targets-of-diseases.pdf`)

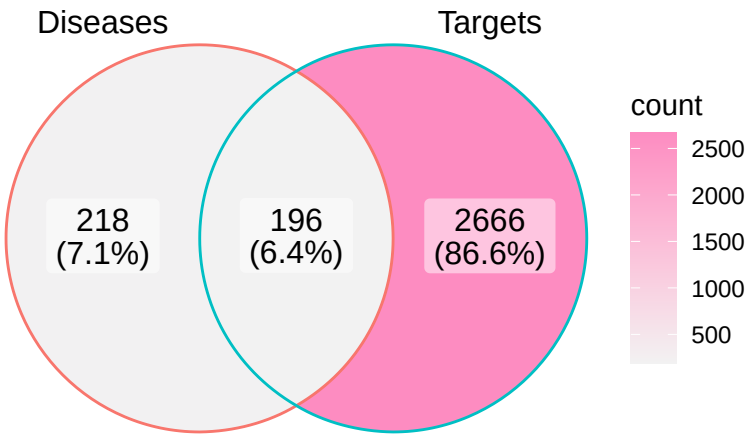

Figure 4: Targets intersect with targets of diseases

Intersection :

IL10, HMOX1, MMP9, PTGS2, SOD2, MPO, NOS2, IL6, CAT, CXCL2, TLR4, ALOX5, RELA, CCL2, CASP3, SELE, XDH, FOS, EDN1, TLR2, PLAT, PTEN, MAPK8, PPARA, CDKN1A, KDR, ADORA2A, CXCL1, PLAU, BCL2, SOD1, PPARG, NOS3, TNF, IL1B, MAPK9, ICAM1, TERT, JUN, ADORA2B, EFNB2, HGF, CD36, IRAK3, SLPI, IL12A, CXCL8, C...

(上述信息框内容已保存至 `Figure+Table/Targets-intersect-with-targets-of-diseases-content`)

6.1.5 PPI 网络

Figure 5 (下方图) 为图 HERBS raw PPI network 概览。

(对应文件为 `Figure+Table/HERBS-raw-PPI-network.pdf`)

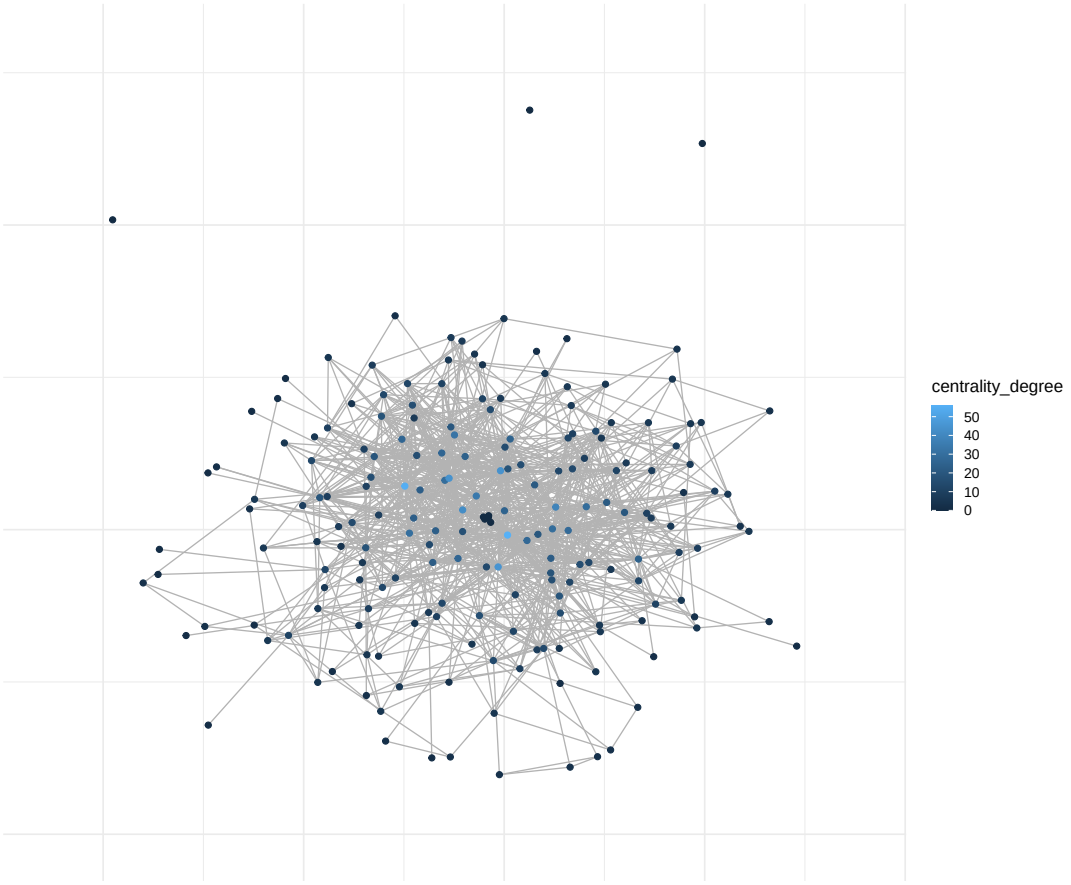

Figure 5: HERBS raw PPI network

Figure 6 (下方图) 为图 HERBS Top30 MCC score 概览。

(对应文件为 `Figure+Table/HERBS-Top30-MCC-score.pdf`)

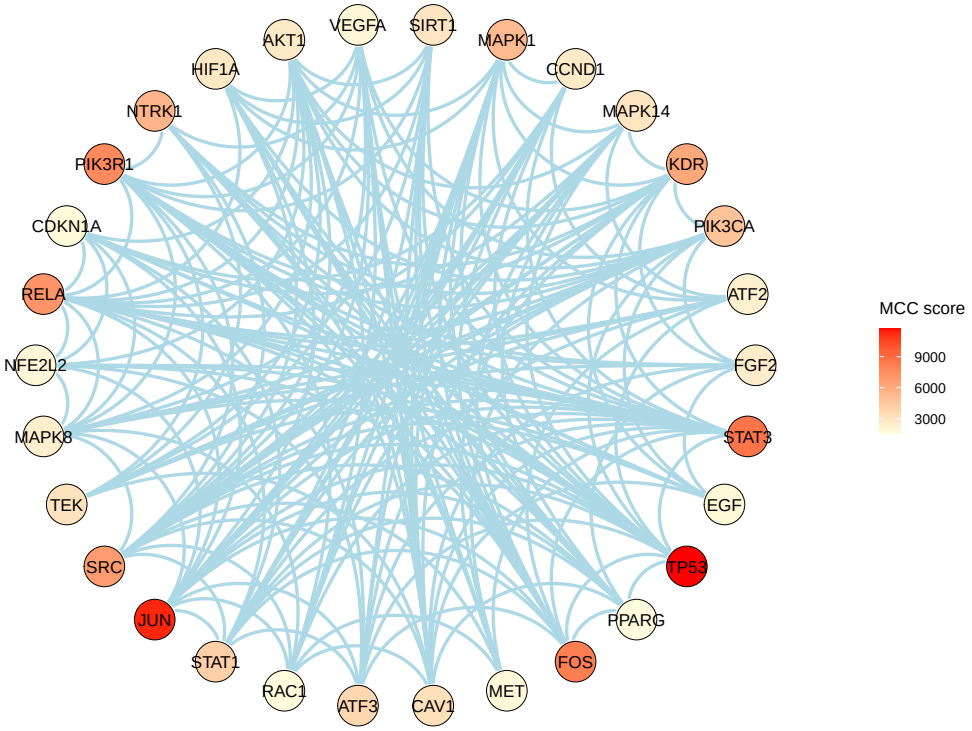

Figure 6: HERBS Top30 MCC score

6.1.6 富集分析 (Top30)

Figure 7 (下方图) 为图 HERBS KEGG enrichment 概览。

(对应文件为 `Figure+Table/HERBS-KEGG-enrichment.pdf`)

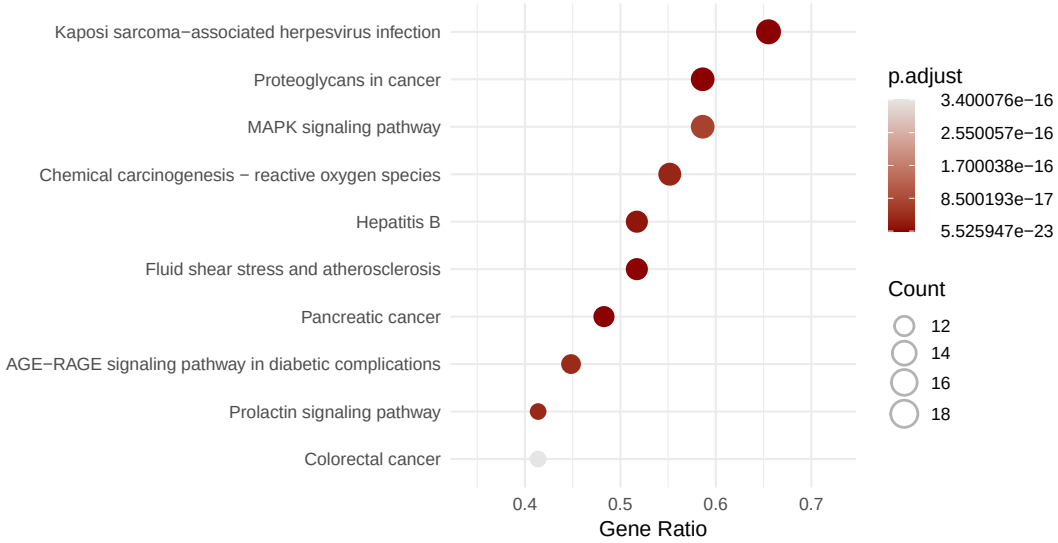

Figure 7: HERBS KEGG enrichment

Table 5 (下方表格) 为表格 HERBS KEGG enrichment data 概览。

(对应文件为 `Figure+Table/HERBS-KEGG-enrichment-data.xlsx`)

> 注：表格共有 181 行 9 列，以下预览的表格可能省略部分数据；表格含有 181 个唯一 'ID'。

1. pvalue: 显著性 P。

Table 5: HERBS KEGG enrichment data

ID	Descri…	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa05167	Kaposi…	19/29	194/8661	3.0530…	5.5259…	1.0605…	207/59…	19
hsa05212	Pancre…	14/29	76/8661	3.1769…	2.8751…	5.5178…	207/59…	14
hsa05205	Proteo…	17/29	205/8661	4.7725…	2.8794…	5.5261…	207/85…	17
hsa05418	Fluid …	15/29	139/8661	3.5822…	1.6209…	3.1109…	207/85…	15
hsa05161	Hepati…	15/29	162/8661	3.8556…	1.3957…	2.6786…	207/13…	15
hsa05208	Chemic…	16/29	223/8661	1.1000…	3.3185…	6.3688…	207/19…	16
hsa04917	Prolac…	12/29	70/8661	1.3458…	3.4799…	6.6785…	207/59…	12
hsa04933	AGE-RA…	13/29	100/8661	1.6908…	3.8255…	7.3418…	207/59…	13
hsa04010	MAPK s…	17/29	301/8661	3.5870…	7.2139…	1.3844…	207/13…	17
hsa05210	Colore…	12/29	86/8661	1.8784…	3.4000…	6.5252…	207/59…	12
hsa05235	PD-L1 …	12/29	89/8661	2.9003…	4.4268…	8.4958…	207/19…	12
hsa05417	Lipid …	15/29	215/8661	2.9349…	4.4268…	8.4958…	207/23…	15
hsa04151	PI3K-A…	17/29	359/8661	7.1506…	9.9559…	1.9106…	207/13…	17
hsa01522	Endocr…	12/29	98/8661	9.7631…	1.2622…	2.4224…	207/59…	12
hsa01521	EGFR t…	11/29	79/8661	5.3600…	6.2132…	1.1924…	207/19…	11
…	…	…	…	…	…	…	…	…

6.1.7 CIR 的 GEO 数据差异分析

Data Source ID :

GSE163614

data_processing :

paired-end reads were harvested from Illumina NovaSeq 6000 sequencer, and were quality controlled by Q30.

data_processing.1 :

After 3’ adaptor-trimming and low quality reads removing by cutadapt software (v1.9.3), the high quality trimmed reads were aligned to the rat reference genome (UCSC RN5).

data_processing.2 :

Then, guided by the Ensembl gtf gene annotation file with hisat2 software (v2.0.4), cuffdiff software (v2.2.1, part of cufflinks) was used to get the gene level FPKM as the expression profiles of mRNA, and fold change and p-value were calculated based on FPKM, differentially expressed mRNA were i...

data_processing.3 :

Genome_build: UCSC RN5

(Others) :

...

Table 6 (下方表格) 为表格 RAT metadata 概览。

(对应文件为 `Figure+Table/RAT-metadata.csv`)

注：表格共有 6 行 9 列，以下预览的表格可能省略部分数据；表格含有 6 个唯一 ‘sample’。

1. sample: 样品名称
2. group: 分组名称

Table 6: RAT metadata

sample	group	lib.size	norm.f...	rownames	title	strain...	time.p...	tissue...
MCAO1	Model	523780...	1	GSM498...	MCAO/R-1	Spragu...	24 h	brain
MCAO2	Model	531002...	1	GSM498...	MCAO/R-2	Spragu...	24 h	brain
MCAO3	Model	582734...	1	GSM498...	MCAO/R-3	Spragu...	24 h	brain
Sham1	Control	599207...	1	GSM498...	Sham-1	Spragu...	24 h	brain
Sham2	Control	585317...	1	GSM498...	Sham-2	Spragu...	24 h	brain
Sham3	Control	588288...	1	GSM498...	Sham-3	Spragu...	24 h	brain

6.1.7.1 差异分析

Figure 8 (下方图) 为图 RAT Model vs Control DEGs 概览。

(对应文件为 `Figure+Table/RAT-Model-vs-Control-DEGs.pdf`)

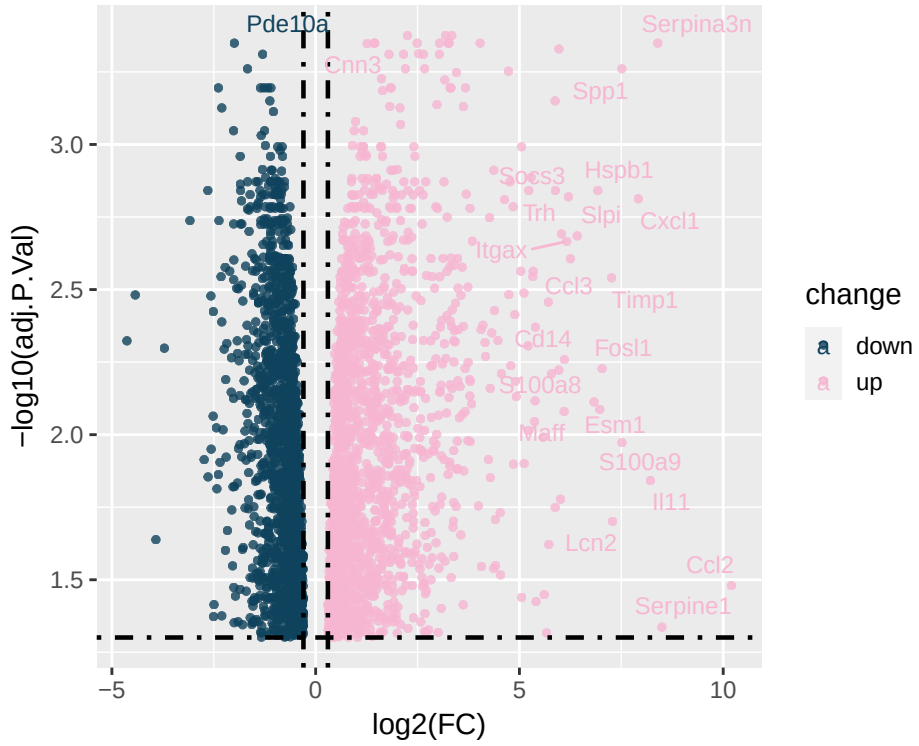

Figure 8: RAT Model vs Control DEGs

6.1.7.2 由大鼠基因映射到人类基因

使用 biomart 将基因映射。

Table 7 (下方表格) 为表格 RAT Mapped DEGs 概览。

(对应文件为 `Figure+Table/RAT-Mapped-DEGs.tsv`)

注：表格共有 2921 行 23 列，以下预览的表格可能省略部分数据；表格含有 2921 个唯一 'hgnc_symbol'。

1. hgnc_symbol: 基因名 (Human)
2. pathway: 相关通路。
3. logFC: estimate of the log2-fold-change corresponding to the effect or contrast (for 'topTableF' there may be several columns of log-fold-changes)
4. AveExpr: average log2-expression for the probe over all arrays and channels, same as 'Amean' in the 'MarrayLM' object
5. t: moderated t-statistic (omitted for 'topTableF')
6. P.Value: raw p-value
7. B: log-odds that the gene is differentially expressed (omitted for 'topTreat')
8. gene_id: GENCODE/Ensembl gene ID
9. strand: genomic strand

Table 7: RAT Mapped DEGs

hgnc_s…	rgd_sy…	rownames	gene_id	gene_s…	biotype	strand	locus	Synonyms	dbXrefs
GPNMB	Gpnmb	4927	ENSRNO…	Gpnmb	protei…	+	chr4:1…	-	RGD:71…
PDPN	Pdpn	8530	ENSRNO…	Pdpn	protei…	-	chr5:1…	E11|Gp…	RGD:61…
STAT3	Stat3	11467	ENSRNO…	Stat3	protei…	-	chr10:…	-	RGD:37…
CNN3	Cnn3	6554	ENSRNO…	Cnn3	protei…	+	chr2:2…	-	RGD:71…
DDX21	Ddx21	18611	ENSRNO…	Ddx21	protei…	-	chr20:…	Ddx21a…	RGD:13…
FLNC	Flnc	4001	ENSRNO…	Flnc	protei…	+	chr4:5…	ABP-L|…	RGD:13…
IGFBP3	Igfbp3	4835	ENSRNO…	Igfbp3	protei…	-	chr14:…	IGF-BP3	RGD:28…
MMP9	Mmp9	10085	ENSRNO…	Mmp9	protei…	+	chr3:1…	-	RGD:62…
PDE10A	Pde10a	6404	ENSRNO…	Pde10a	protei…	-	chr1:5…	Pde10a3	RGD:68…
SBNO2	Sbno2	7959	ENSRNO…	Sbno2	protei…	+	chr7:1…	RGD130…	RGD:13…
SERPINA3	Serpina3n	5928	ENSRNO…	Serpina3n	protei…	+	chr6:1…	CPi-26…	RGD:37…
CSF2RB	Csf2rb	83	ENSRNO…	Csf2rb	protei…	+	chr7:1…	Csf2rb1	RGD:62…
FLNA	Flna	17331	ENSRNO…	Flna	protei…	+	chr1:1…	RGD156…	RGD:15…
LCP1	Lcp1	5808	ENSRNO…	Lcp1	protei…	+	chr15:…	-	RGD:13…
MAST3	Mast3	12964	ENSRNO…	Mast3	protei…	+	chr16:…	-	RGD:15…
…	…	…	…	…	…	…	…	…	…

6.1.7.3 富集分析

Figure 9 (下方图) 为图 MAP KEGG enrichment 概览。

(对应文件为 `Figure+Table/MAP-KEGG-enrichment.pdf`)

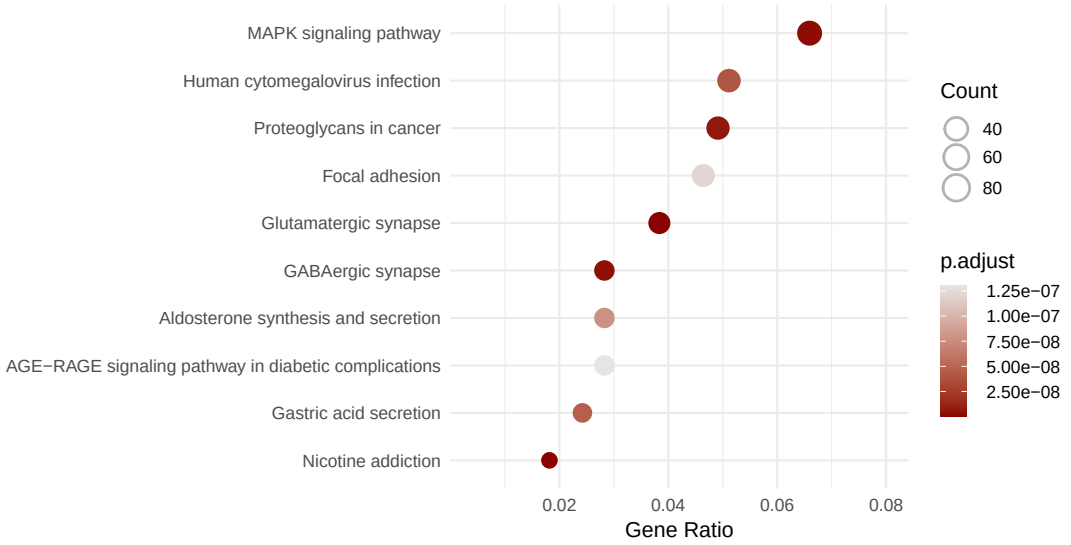

Figure 9: MAP KEGG enrichment

Table 8 (下方表格) 为表格 MAP KEGG enrichment data 概览。

(对应文件为 `Figure+Table/MAP-KEGG-enrichment-data.xlsx`)

> 注：表格共有 337 行 9 列，以下预览的表格可能省略部分数据；表格含有 337 个唯一 'ID'。

1. pvalue: 显著性 P。

Table 8: MAP KEGG enrichment data

ID	Descri…	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa04724	Glutam…	57/1486	115/8661	9.1917…	3.0976…	1.6641…	107/19…	57
hsa05033	Nicoti…	27/1486	40/8661	2.0977…	3.5346…	1.8989…	773/77…	27
hsa04010	MAPK s…	98/1486	301/8661	2.5161…	2.8264…	1.5185…	10000/…	98
hsa04727	GABAer…	42/1486	89/8661	4.4346…	3.7361…	2.0072…	18/107…	42
hsa05205	Proteo…	73/1486	205/8661	9.9744…	6.7227…	3.6117…	60/71/…	73
hsa05163	Human …	76/1486	225/8661	7.0361…	3.9519…	2.1231…	107/19…	76
hsa04971	Gastri…	36/1486	76/8661	9.5518…	4.5985…	2.4705…	60/71/…	36
hsa04925	Aldost…	42/1486	98/8661	1.8482…	7.7857…	4.1828…	107/19…	42
hsa04510	Focal …	69/1486	203/8661	3.2209…	1.2060…	6.4794…	60/71/…	69
hsa04933	AGE-RA…	42/1486	100/8661	3.8738…	1.3054…	7.0136…	183/10…	42
hsa04015	Rap1 s…	70/1486	210/8661	6.3009…	1.8176…	9.7653…	60/71/…	70
hsa05032	Morphi…	39/1486	91/8661	7.0065…	1.8176…	9.7653…	107/19…	39
hsa04360	Axon g…	63/1486	182/8661	7.0117…	1.8176…	9.7653…	655/65…	63
hsa04611	Platel…	48/1486	124/8661	7.7305…	1.8608…	9.9974…	60/71/…	48
hsa04670	Leukoc…	45/1486	115/8661	1.5365…	3.4520…	1.8546…	60/71/…	45

ID	Descri...	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
...	...	...	...	...	...	...	...	...

可以发现，‘MARK’ 通路居于首位。以下展示 Fig. 7 富集结果的 ‘MARK’ 通路：

Figure 10 (下方图) 为图 HERBS hsa04010 visualization 概览。

(对应文件为 `Figure+Table/hsa04010.pathview.png`)

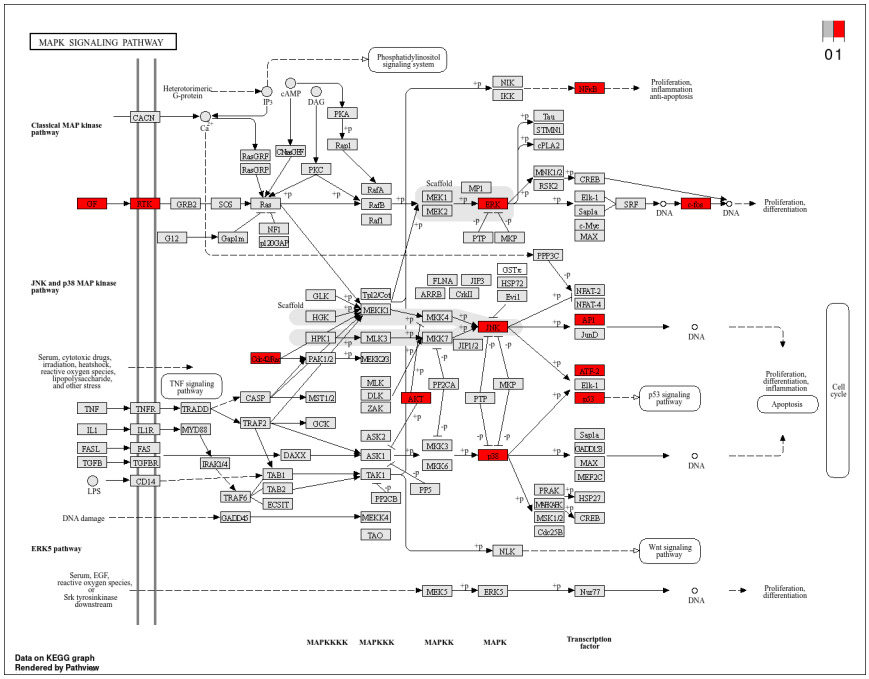

Figure 10: HERBS hsa04010 visualization

Interactive figure :

https://www.genome.jp/pathway/hsa04010

6.1.8 复方靶点通路与 CIR DEGs 富集结果的共同富集通路

Table 9 (下方表格) 为表格 HERBS pathways intersection 概览。

(对应文件为 `Figure+Table/HERBS-pathways-intersection.xlsx`)

注：表格共有 99 行 9 列，以下预览的表格可能省略部分数据；表格含有 99 个唯一 ‘ID’。

1. pvalue: 显著性 P。

Table 9: HERBS pathways intersection

ID	Descri…	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa05167	Kaposi…	19/29	194/8661	3.0530…	5.5259…	1.0605…	207/59…	19
hsa05212	Pancre…	14/29	76/8661	3.1769…	2.8751…	5.5178…	207/59…	14
hsa05205	Proteo…	17/29	205/8661	4.7725…	2.8794…	5.5261…	207/85…	17
hsa05418	Fluid …	15/29	139/8661	3.5822…	1.6209…	3.1109…	207/85…	15
hsa05161	Hepati…	15/29	162/8661	3.8556…	1.3957…	2.6786…	207/13…	15
hsa04933	AGE-RA…	13/29	100/8661	1.6908…	3.8255…	7.3418…	207/59…	13
hsa04010	MAPK s…	17/29	301/8661	3.5870…	7.2139…	1.3844…	207/13…	17
hsa05210	Colore…	12/29	86/8661	1.8784…	3.4000…	6.5252…	207/59…	12
hsa05417	Lipid …	15/29	215/8661	2.9349…	4.4268…	8.4958…	207/23…	15
hsa04151	PI3K-A…	17/29	359/8661	7.1506…	9.9559…	1.9106…	207/13…	17
hsa01522	Endocr…	12/29	98/8661	9.7631…	1.2622…	2.4224…	207/59…	12
hsa04510	Focal …	14/29	203/8661	5.4923…	6.2132…	1.1924…	207/85…	14
hsa05207	Chemic…	14/29	212/8661	1.0132…	1.0787…	2.0703…	207/13…	14
hsa05163	Human …	14/29	225/8661	2.3412…	2.3542…	4.5181…	207/13…	14
hsa04926	Relaxi…	12/29	129/8661	2.9702…	2.8295…	5.4304…	207/13…	12
…	…	…	…	…	…	…	…	…

6.1.9 复方对 MARK 通路

Figure 11 (下方图) 为图 Network pharmacology target MARK 概览。

(对应文件为 `Figure+Table/Network-pharmacology-target-MARK.pdf`)

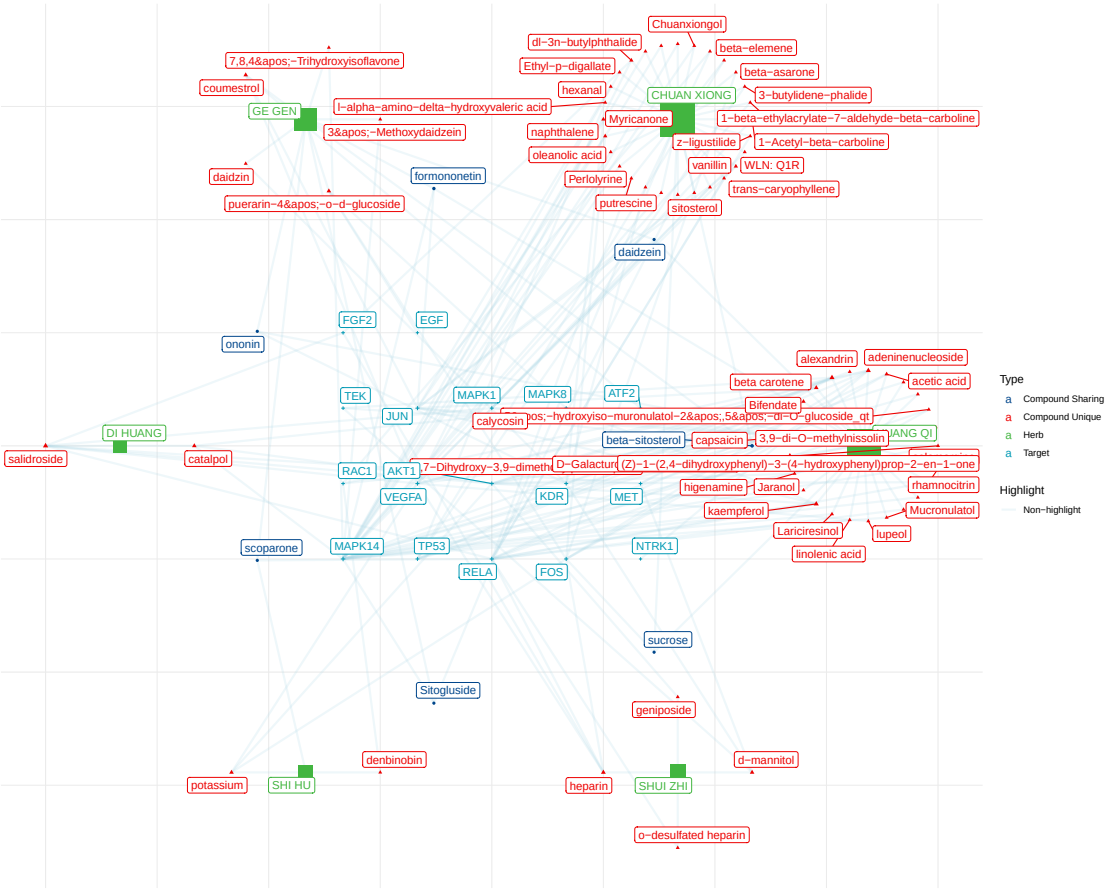

Figure 11: Network pharmacology target MARK

6.1.9.1 复方作用于 MARK 通路的成分

Table 10 (下方表格) 为表格 Network pharmacology target MARK data 概览。

(对应文件为 `Figure+Table/Network-pharmacology-target-MARK-data.xlsx`)

> 注：表格共有 297 行 3 列，以下预览的表格可能省略部分数据；表格含有 6 个唯一 'Herb_pinyin_name'。

Table 10: Network pharmacology target MARK data

Herb_pinyin_name	Ingredient.name	Target.name
HUANG QI	1,7-Dihydroxy-3,9-dimethoxy...	MAPK14
CHUAN XIONG	1-Acetyl-beta-carboline	MAPK14
CHUAN XIONG	1-beta-ethylacrylate-7-alde...	MAPK14
HUANG QI	3,9-di-O-methylnissolin	MAPK14
CHUAN XIONG	3-butylidene-phalide	TP53
GE GEN	3'-Methoxydaidzein	MAPK14
HUANG QI	5'-hydroxyiso-muronula...	RELA

Herb_pinyin_name	Ingredient.name	Target.name
HUANG QI	(6aR,11aR)-9,10-dimethoxy-6...	MAPK14
GE GEN	7,8,4'-Trihydroxyisofl...	MAPK14
HUANG QI	7-O-methylisomucronulatol	MAPK14
HUANG QI	acetic acid	FOS
HUANG QI	acetic acid	RELA
HUANG QI	acetic acid	FOS
HUANG QI	acetic acid	RELA
HUANG QI	adeninenucleoside	FOS
...	...	...

6.2 水蛭素 Hirudin

6.2.1 Hirudin 靶点 (获取更多靶点)

HERBs 数据库包含的 Hirudin 靶点较少:

Table 11 (下方表格) 为表格 Hirudin targets in HERB database 概览。

(对应文件为 `Figure+Table/Hirudin-targets-in-HERB-database.csv`)

> 注: 表格共有 4 行 3 列, 以下预览的表格可能省略部分数据; 表格含有 1 个唯一 'Herb_pinyin_name'。

Table 11: Hirudin targets in HERB database

Herb_pinyin_name	Ingredient.name	Target.name
SHUI ZHI	hirudin	F2
SHUI ZHI	hirudin	F3
SHUI ZHI	hirudin	F5
SHUI ZHI	hirudin	MIF

6.2.1.1 GeneCards 获取化合物靶点

bindingdb, drugbank, 以及预测工具 Super-Pred 等都难以获取更多关于 hirudin 靶点信息。因此, 这里使用 `GeneCards` 搜索。

The GeneCards data was obtained by querying :

hirudin

Restrict (with quotes) :

FALSE

Filtering by Score: :

Score > 0

Advance search: :

[compounds] (hirudin)

Table 12 (下方表格) 为表格 Hirudin targets from GeneCards 概览。

(对应文件为 `Figure+Table/Hirudin-targets-from-GeneCards.xlsx`)

注：表格共有 45 行 7 列，以下预览的表格可能省略部分数据；表格含有 45 个唯一 'Symbol'。

Table 12: Hirudin targets from GeneCards

Symbol	Description	Category	UniProt_ID	GIFtS	GC_id	Score
F2	Coagulatio…	Protein Co…	P00734	58	GC11P047386	2.58
F2R	Coagulatio…	Protein Co…	P25116	55	GC05P076716	2.23
F10	Coagulatio…	Protein Co…	P00742	58	GC13P113122	1.76
FGA	Fibrinogen…	Protein Co…	P02671	58	GC04M154583	1.76
PLAT	Plasminoge…	Protein Co…	P00750	57	GC08M042174	1.76
F3	Coagulatio…	Protein Co…	P13726	54	GC01M094825	1.76
PLG	Plasminogen	Protein Co…	P00747	58	GC06P160702	1.59
CPA1	Carboxypep…	Protein Co…	P15085	51	GC07P130380	1.12
PLAU	Plasminoge…	Protein Co…	P00749	60	GC10P073909	0.64
SERPINE1	Serpin Fam…	Protein Co…	P05121	59	GC07P101127	0.64
CCL2	C-C Motif …	Protein Co…	P13500	58	GC17P034255	0.64
CD40LG	CD40 Ligand	Protein Co…	P29965	58	GC0XP136649	0.64
CD55	CD55 Molec…	Protein Co…	P08174	58	GC01P207321	0.64
SERPINC1	Serpin Fam…	Protein Co…	P01008	58	GC01M174899	0.64
TBXA2R	Thromboxan…	Protein Co…	P21731	58	GC19M003594	0.64
…	…	…	…	…	…	…

6.2.2 Hirudin 靶点与 CIR DEGs 交集

Figure 12 (下方图) 为图 Intersection of Hirudin Targets with CIR DEGs 概览。

(对应文件为 `Figure+Table/Intersection-of-Hirudin-Targets-with-CIR-DEGs.pdf`)

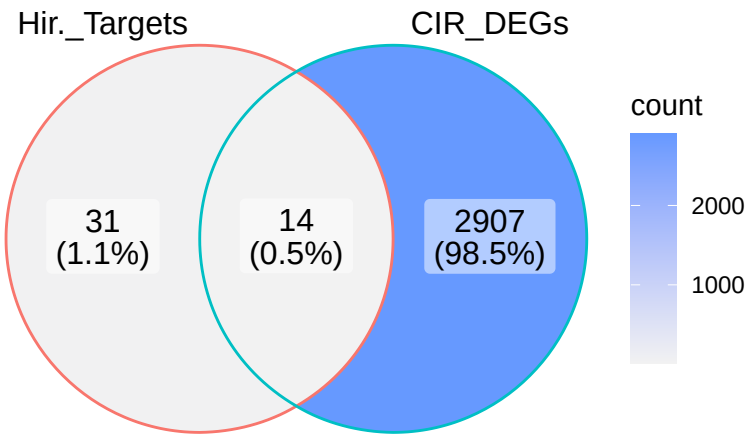

Figure 12: Intersection of Hirudin Targets with CIR DEGs

Intersection :

PLAT, PLAU, SERPINE1, VWF, THBD, SELP, THBS1, TIMP1, PLAUR, F2RL1, SELE, PROCR, FGL2, SCG5

(上述信息框内容已保存至 `Figure+Table/Intersection-of-Hirudin-Targets-with-CIR-DEGs-content`)

6.2.2.1 交集基因的富集分析

Figure 13 (下方图) 为图 HIRUDIN CIR KEGG enrichment 概览。

(对应文件为 `Figure+Table/HIRUDIN-CIR-KEGG-enrichment.pdf`)

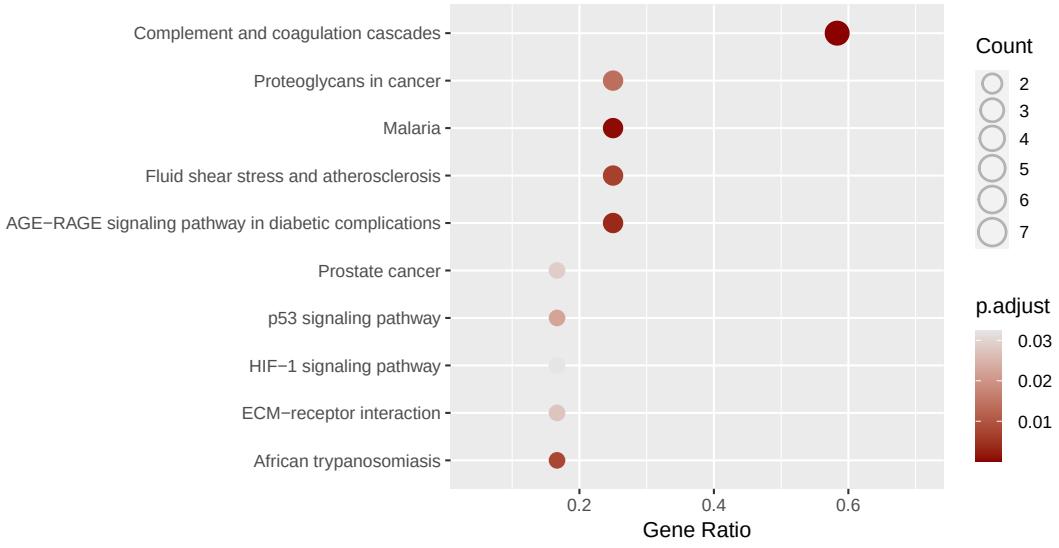

Figure 13: HIRUDIN CIR KEGG enrichment

6.2.2.2 与复方共同作用的信号通路

因为在 Hirudin 的富集分析前，额外从 GeneCards 获取了 Hirudin 的靶点，这一部分在复方分析中是不包含的；因此，这里尝试寻找它们共同的靶向通路 (复方与获取了额外靶点的 Hirudin 的共同富集通路)。

Table 13 (下方表格) 为表格 HIRUDIN Herbs pathways intersection 概览。

(对应文件为 `Figure+Table/HIRUDIN-Herbs-pathways-intersection.csv`)

> 注：表格共有 7 行 9 列，以下预览的表格可能省略部分数据；表格含有 7 个唯一 'ID'。

1. pvalue: 显著性 P。

Table 13: HIRUDIN Herbs pathways intersection

ID	Descri…	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa04933	AGE-RA…	3/12	100/8661	0.0003…	0.0034…	0.0018…	6401/5…	3
hsa05418	Fluid …	3/12	139/8661	0.0008…	0.0068…	0.0035…	5327/6…	3
hsa05205	Proteo…	3/12	205/8661	0.0024…	0.0139…	0.0073…	5328/5…	3
hsa04115	p53 si…	2/12	75/8661	0.0046…	0.0224…	0.0118…	5054/7057	2
hsa05215	Prosta…	2/12	97/8661	0.0076…	0.0287…	0.0151…	5327/5328	2
hsa04066	HIF-1 …	2/12	109/8661	0.0095…	0.0324…	0.0170…	5054/7076	2
hsa04371	Apelin…	2/12	139/8661	0.0151…	0.0469…	0.0247…	5327/5054	2

6.3 最终筛选 (着重考虑 Hirudin)

为了缩小可选通路范围，这里尝试将以下的富集结果取共同的交集 (已在上述部分完成)：

- 复方靶向 CIR (靶点来源见 Fig. 2) 的通路 (富集见 Fig. 7)
- GEO 数据集 (GSE163614) CIR DEGs 的富集结果的通路 (富集见 Fig. 9)
- 获取了更多靶点信息 (因为 HERBS 数据库或其他数据库包含的靶点信息太少，不利于分析) 的 Hirudin 靶向 CIR (GEO DEGs) 的基因的富集分析 (Fig. 13)

得到 (去除了名称包含其他疾病的通路)：

Table 14 (下方表格) 为表格 All pathways intersection 概览。

(对应文件为 `Figure+Table/All-pathways-intersection.csv`)

> 注：表格共有 2 行 9 列，以下预览的表格可能省略部分数据；表格含有 2 个唯一 'ID'。

1. pvalue: 显著性 P。

Table 14: All pathways intersection

ID	Descri...	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa04066	HIF-1 ...	11/29	109/8661	2.1482...	1.4400...	2.7637...	207/10...	11
hsa04371	Apelin...	3/29	139/8661	0.0108...	0.0141...	0.0027...	207/59...	3

6.3.1 复方对筛选通路的靶向

Figure 14 (下方图) 为图 HERBS hsa04066 visualization 概览。

(对应文件为 `Figure+Table/hsa04066.pathview.png`)

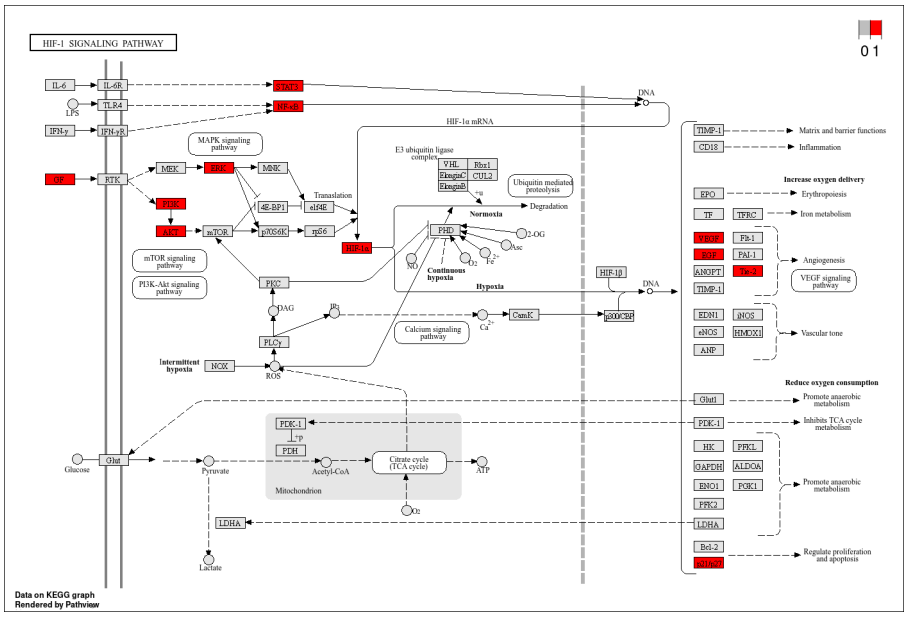

Figure 14: HERBS hsa04066 visualization

Interactive figure :

https://www.genome.jp/pathway/hsa04066

Figure 15 (下方图) 为图 HERBS hsa04371 visualization 概览。

(对应文件为 `Figure+Table/hsa04371.pathview.png`)

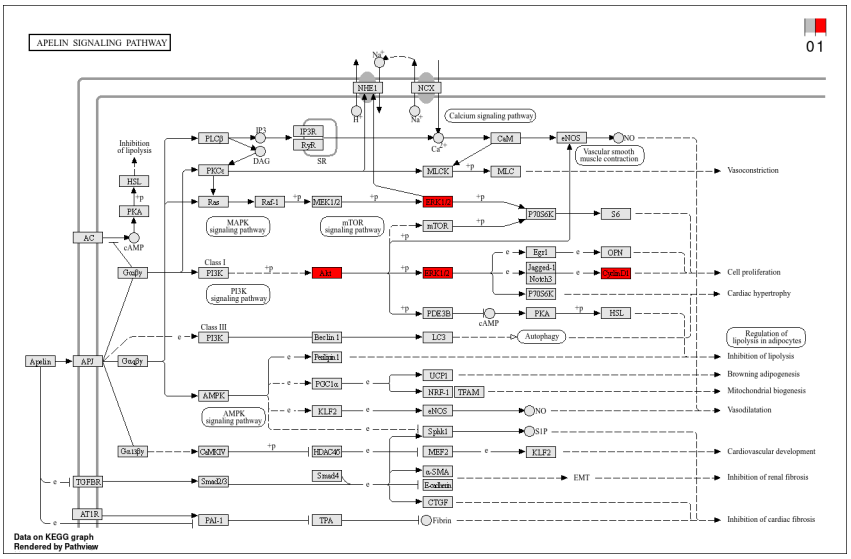

Figure 15: HERBS hsa04371 visualization

Interactive figure :

https://www.genome.jp/pathway/hsa04371

6.3.1.1 相关成分

Table 15 (下方表格) 为表格 Compounds target HIF 1 signaling pathway 概览。

(对应文件为 `Figure+Table/Compounds-target-HIF-1-signaling-pathway.xlsx`)

注：表格共有 137 行 9 列，以下预览的表格可能省略部分数据；表格含有 38 个唯一 'Ingredient.id'。

Table 15: Compounds target HIF 1 signaling pathway

Ingred……1	Herb_p…	Ingred……3	Ingred……4	Target.id	Target…	Databa…	Paper.id	…
HBIN00…	CHUAN …	3-buty…	NA	HBTAR0…	CDKN1A	NA	NA	…
HBIN01…	HUANG QI	5&apos…	NA	HBTAR0…	RELA	NA	NA	…
HBIN01…	HUANG QI	acetic…	AI3-02…	HBTAR0…	RELA	NA	NA	…

Ingred......1	Herb_p...	Ingred......3	Ingred......4	Target.id	Target...	Databa...	Paper.id	...
HBIN01...	HUANG QI	acetic...	AI3-02...	HBTAR0...	RELA	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	HIF1A	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	VEGFA	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	HIF1A	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	VEGFA	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	HIF1A	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	VEGFA	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	HIF1A	NA	NA	...
HBIN01...	HUANG QI	adenin...	NA	HBTAR0...	VEGFA	NA	NA	...
HBIN01...	HUANG QI	astram...	AC1L3V...	HBTAR0...	AKT1	NA	NA	...
HBIN01...	HUANG QI	astram...	AC1L3V...	HBTAR0...	TEK	NA	NA	...
HBIN01...	HUANG QI	beta c...	Spectr...	HBTAR0...	AKT1	NA	NA	...
...	...	...	...	...	...	...	...	...

Table 16 (下方表格) 为表格 Compounds target Apelin signaling pathway 概览。

(对应文件为 `Figure+Table/Compounds-target-Apelin-signaling-pathway.xlsx`)

> 注：表格共有 61 行 9 列，以下预览的表格可能省略部分数据；表格含有 17 个唯一 'Ingredient.id'。

Table 16: Compounds target Apelin signaling pathway

Ingred......1	Herb_p...	Ingred......3	Ingred......4	Target.id	Target...	Databa...	Paper.id	...
HBIN00...	CHUAN ...	3-buty...	NA	HBTAR0...	CCND1	NA	NA	...
HBIN01...	HUANG QI	5&apos...	NA	HBTAR0...	CCND1	NA	NA	...
HBIN01...	HUANG QI	astram...	AC1L3V...	HBTAR0...	AKT1	NA	NA	...
HBIN01...	HUANG QI	beta c...	Spectr...	HBTAR0...	AKT1	NA	NA	...
HBIN01...	HUANG QI	beta c...	Spectr...	HBTAR0...	AKT1	NA	NA	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	AKT1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	MAPK1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	AKT1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	MAPK1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	AKT1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	MAPK1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	AKT1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	MAPK1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	AKT1	NA	HBREF0...	...
HBIN01...	HUANG QI	calycosin	HSDB 8...	HBTAR0...	MAPK1	NA	HBREF0...	...
...	...	...	...	...	...	...	...	...

6.3.2 Hirudin 对筛选通路的靶向

Figure 16 (下方图) 为图 HIRUDIN hsa04066 visualization 概览。

(对应文件为 `Figure+Table/hsa04066.pathview.png`)

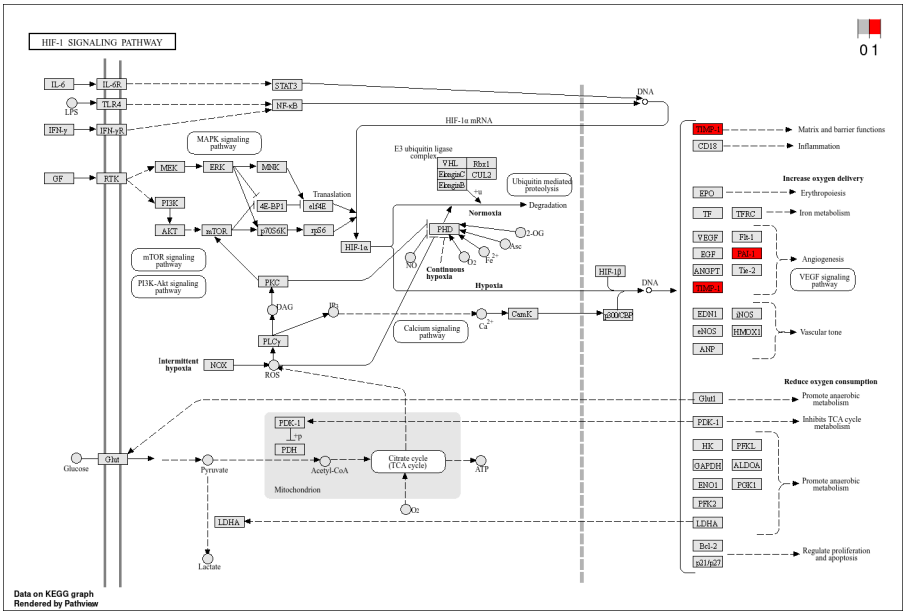

Figure 16: HIRUDIN hsa04066 visualization

Interactive figure :

https://www.genome.jp/pathway/hsa04066

Figure 17 (下方图) 为图 HIRUDIN hsa04371 visualization 概览。

(对应文件为 `Figure+Table/hsa04371.pathview.png`)

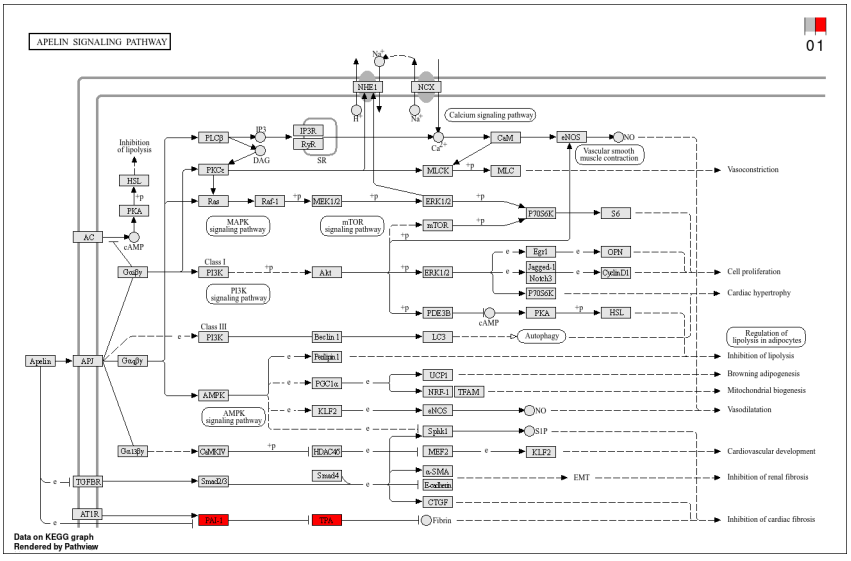

Figure 17: HIRUDIN hsa04371 visualization

Interactive figure :

https://www.genome.jp/pathway/hsa04371

Reference

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