RNA-seq 探究 rTMS 对 SCI 和 NP 的影响

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LiChuang Huang



@ 立效研究院

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1 摘要

质子磁共振光谱法 (H-MRS) 加巴喷丁 (Gabapentin)

不完全性脊髓损伤 (Incomplete spinal cord injury, iSCI) - GSE226238

神经病理性疼痛 (neuropathic pain, NP) - GSE126611

重复经颅磁刺激治疗 (repeat transcranial magnetic stimulation, rTMS) - GSE230150

GEO 有 iSCI、NP、rTMS 各自的基因表达数据集,可以从三者之间的关联性寻找 rTMS 可能的疗效和机制

2 前言

3 材料和方法

3.1 材料

All used GEO expression data and their design:

- **GSE126611**: We investigated n=14 samples, no replicates, comparison between two patient groups, and patient group with healthy controls. (NL-1) is with nerve lesion and (NL-0) is without neuropathic pain.
- **GSE230149**: Gene expression microarray analysis of 48 mouse brain samples, consisting of matched sets of hippocampus (HIPP, N=24) and parietal cortex (PCTX, N=24) from young sham (Y_Sham, N=4), young iTBS (Y_i...
- GSE226238: RNAsequencing from whole blood taken from participants with SCI within 3 days of injury, at 3 MPI, 6 MPI and 12 MPI. Data was compared to un-injured participants as controls. Inclusion and exclusio...

3.2 方法

Mainly used method:

- ClusterProfiler used for gene enrichment analysis.¹
- GEO https://www.ncbi.nlm.nih.gov/geo/ used for expression dataset aquisition .
- Limma and edgeR used for differential expression analysis.^{2,3}
- Other R packages (eg., dplyr and ggplot2) used for statistic analysis or data visualization.

4 分析结果

- 4.1 不完全性脊髓损伤和神经病理性疼痛 (SCI) (NP)
- 4.2 SCI 和 NP 关联分析
- 4.3 重复经颅磁刺激治疗 (rTMS)
- 4.4 rTMS 和 SCI、NP 的关联
- 5 结论
- 6 附:分析流程
- 6.1 不完全性脊髓损伤 (Incomplete spinal cord injury, iSCI) (Human)
- 6.1.1 元数据
 - GSE226238

根据文献提供的数据整理信息4:

Complete: AIS A-B Incomplete: AIS C-D

使用的样本的信息:

Table 1 (下方表格) 为表格 SCI used sample metadata 概览。

(对应文件为 Figure+Table/SCI-used-sample-metadata.xlsx)

注: 表格共有 19 行 12 列,以下预览的表格可能省略部分数据;表格含有 19 个唯一'sample'。

1. sample: 样品名称 2. group: 分组名称

Table 1: SCI used sample metadata

sample	rownames	title	group	tissu	treat	group	id	status	AIS
ID13	GSM70	ID13,	CTL	Whole	NA	control	NA	NA	NA
ID16	GSM70	ID16,	CTL	Whole	NA	control	NA	NA	NA
ID14	GSM70	ID14,	CTL	Whole	NA	control	NA	NA	NA
ID15	GSM70	${ m ID15,}$	CTL	Whole	NA	control	NA	NA	NA
ID17	GSM70	ID17,	CTL	Whole	NA	control	NA	NA	NA
ID1V0	GSM70	${\rm ID1v0}$	SCI	Whole	Acute	sci	1	0	D
ID18	GSM70	ID18,	CTL	Whole	NA	control	NA	NA	NA
ID19	GSM70	ID19,	CTL	Whole	NA	control	NA	NA	NA

sample	rownames	title	group	tissu	treat	group	id	status	AIS
ID1V12	GSM70	ID1v1	SCI	Whole	12mpi	sci	1	12	D
ID20	GSM70	${\rm ID}20,$	CTL	Whole	NA	control	NA	NA	NA
ID1V3	GSM70	ID1v3	SCI	Whole	3mpi	sci	1	3	D
ID1V6	GSM70	ID1v6	SCI	Whole	6mpi	sci	1	6	D
ID21	$\operatorname{GSM70}$	${\rm ID21,}$	CTL	Whole	NA	control	NA	NA	NA
ID2V3	GSM70	${ m ID2v3}$	SCI	Whole	3mpi	sci	2	3	D
ID2V0	$\operatorname{GSM70}$	${\rm ID2v0}$	SCI	Whole	Acute	sci	2	0	D

6.1.2 差异分析

Table 2 (下方表格) 为表格 SCI data DEGs 概览。

(对应文件为 Figure+Table/SCI-data-DEGs.csv)

注: 表格共有 3508 行 7 列,以下预览的表格可能省略部分数据;表格含有 3508 个唯一'rownames'。

- 1. logFC: estimate of the log2-fold-changes corresponding to the effect or contrasts(for 'topTableF' there may be several scolumns of log-fold-changes)
- 2. Ave Expr: average log2-expression for the probesover all arrays and channels, same as s 'Amean' in the 'MarrayLM' object
- 3. t: moderated t-statistic (omitted fors 'topTableF')
- 4. P.Value: raw p-value
- 5. B: log-odds that the gene is differentially sexpressed (omitted for 'topTreat')

Table 2: SCI data DEGs

rownames	$\log FC$	AveExpr	t	P.Value	adj.P	В
SLC66A1	-5.39	-2.85	-22.2	2.059	1.472	24.69
CDKN1C	-6.41	-2.34	-18.8	4.387	1.146	22.02
PGAM1	3.931229	4.391	18.79	4.812	1.146	21.93
KLHL26	-6.30	-2.31	-17.0	2.823	5.046	20.33
48	-5.43	-2.32	-16.3	6.538	9.349	19.56
120	-2.92	1.653	-15.7	1.211	1.313	18.98
DUSP23	-6.94	-1.69	-15.7	1.286	1.313	18.93
HIC1	-5.41	-2.45	-15.1	2.686	2.401	18.24
FAM229A	-5.57	-2.12	-14.6	4.877	3.874	17.68
METTL26	-5.53	-2.19	-13.6	1.714	1.225	16.48
CLTB	5.104	-0.35	12.86	4.864	3.162	15.48
LSP1	5.033352	3.102	12.31	1.052	6.271	14.74

rownames	$\log FC$	AveExpr	t	P.Value	adj.P	В
HADH	-4.96	-2.83	-12.2	1.153	6.345	14.65
METTL21A	-4.65	-2.82	-12.1	1.299	6.638	14.53
LRRC24	-4.27	-2.48	-12.0	1.540	7.125	14.37

Figure 1 (下方图) 为图 SCI sci vs control DEGs 概览。

(对应文件为 Figure+Table/SCI-sci-vs-control-DEGs.pdf)

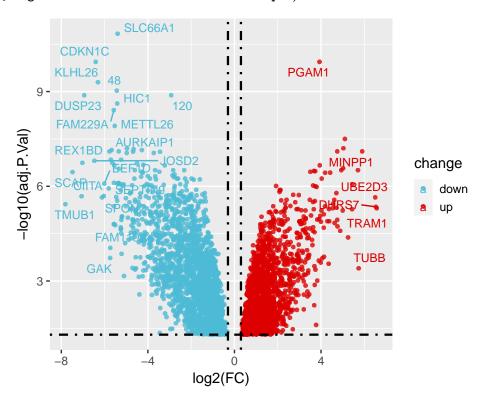


Figure 1: SCI sci vs control DEGs $\,$

6.2 神经病理性疼痛 (neuropathic pain, NP) (Human)

6.2.1 元数据

Table 3 (下方表格) 为表格 NP metadata 概览。

(对应文件为 Figure+Table/NP-metadata.csv)

注: 表格共有 14 行 7 列,以下预览的表格可能省略部分数据;表格含有 14 个唯一'rownames'。

1. sample: 样品名称

2. group: 分组名称

Table 3: NP metadata

-						
rownames	group	lib.size	norm	sample	title	tissu
Contr	Control	37694	0.993	Contr	Contr	white
Contr	Control	35123	0.984	Contr	Contr	white
Contr	Control	40623	1.038	Contr	Contr	white
Contr	Control	31254	0.900	Contr	Contr	white
Contr	Control	36785	1.092	Contr	Contr	white
NL.0	NL.0	39063	0.998	NL.0	NL-0	white
NL.0	NL.0	30209	0.838	NL.0	NL-0	white
NL.0	NL.0	33541	0.873	NL.0	NL-0	white
NL.0	NL.0	43997	1.105	NL.0	NL-0	white
NL.1	NL.1	35856	0.947	NL.1	NL-1	white
NL.1	NL.1	47176	1.217	NL.1	NL-1	white
NL.1	NL.1	33852	0.947	NL.1	NL-1	white
NL.1	NL.1	37527	0.991	NL.1	NL-1	white
NL.1	NL.1	45702	1.142	NL.1	NL-1	white

6.2.2 差异分析

Table 4 (下方表格) 为表格 NP data NL 1 vs Control DEGs 概览。

(对应文件为 Figure+Table/NP-data-NL-1-vs-Control-DEGs.xlsx)

注: 表格共有 263 行 17 列,以下预览的表格可能省略部分数据;表格含有 263 个唯一'rownames'。

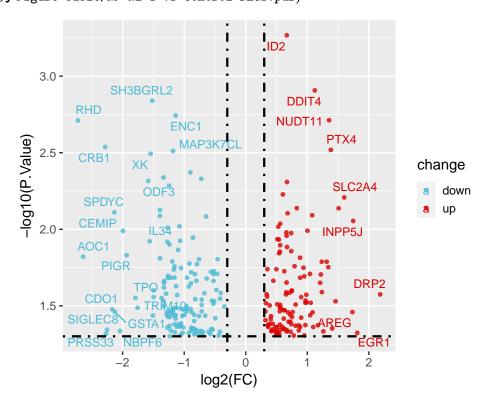
- 1. Chr: 染色体
- 2. Start: 起始点
- 3. End: 结束点
- 4. symbol: 基因或蛋白符号。
- 5. logFC: estimate of the log2-fold-changes corresponding to the effect or contrasts(for 'topTableF' there may be several scolumns of log-fold-changes)
- 6. AveExpr: average log2-expression for the probesover all arrays and channels, same ass 'Amean' in the 'MarrayLM' object
- 7. t: moderated t-statistic (omitted fors 'topTableF')
- 8. P.Value: raw p-value
- 9. B: log-odds that the gene is differentially sexpressed (omitted for 'topTreat')

Table 4: NP data NL 1 vs Control DEGs

rownames	symbol	Chr	Source	Feature	Start	End	Frame	Strand	V8
1992	ID2	chr2	HAVANA	gene	8678845	8684453	0	+	0
9282	DDIT4	chr10	HAVANA	gene	72273920	72276036	0	+	0
17418	MAP3K7CL	chr21	HAVANA	gene	29077471	29175889	0	+	0
5167	ENC1	chr5	HAVANA	gene	74627406	74641424	0	-	0
14324	PER1	chr17	HAVANA	gene	8140472	8156506	0	-	0
6603	TNFAIP3	chr6	HAVANA	gene	13786	13788	0	+	0
15786	PDE4A	chr19	HAVANA	gene	10416773	10469631	0	+	0
14577	CCL4	chr17	HAVANA	gene	36103590	36105621	0	+	0
4463	HOPX	chr4	HAVANA	gene	56647988	56681899	0	-	0
10202	MS4A14	chr11	HAVANA	gene	60378530	60417756	0	+	0
12419	PTGDR	chr14	HAVANA	gene	52267713	52276724	0	+	0
6372	SH3BGRL2	chr6	HAVANA	gene	79631283	79703659	0	+	0
5266	PAM	chr5	HAVANA	gene	10275	10303	0	+	0
4533	PPBP	chr4	HAVANA	gene	73987038	73988197	0	-	0
6909	MTURN	chr7	HAVANA	gene	30134810	30162762	0	+	0
		•••					•••	•••	

Figure 2 (下方图) 为图 NP NL 1 vs Control DEGs 概览。

(对应文件为 Figure+Table/NP-NL-1-vs-Control-DEGs.pdf)



6.3 SCI 和 NP 关联分析

6.3.1 共同差异基因 coDEGs

Figure 3 (下方图) 为图 SCI NP coDEGs 概览。

(对应文件为 Figure+Table/SCI-NP-coDEGs.pdf)

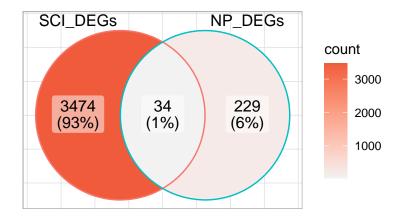


Figure 3: SCI NP coDEGs

Intersection:

PDE4A, ADA, LIMS1, FLYWCH2, LDLR, GOLGA8N, NUDT2, CNTLN, CTSS, KNOP1, PRF1, MAP3K7CL, CLDN5, SHISA8, CMC1, SLAMF8, ELOVL7, AIFM3, GNGT2, PPT1, DAPP1, AOC1, PSMC1, PF4, CBLN3, LPAR6, CPM, EGF, SH3PXD2A, SH3YL1, ATF3, PTCRA, SMIM1, EVI2A

(上述信息框内容已保存至 Figure+Table/SCI-NP-coDEGs-content)

6.3.2 SCI 的 coDEGs 的关联性分析

Figure 4 (下方图) 为图 SCI genes correlation heatmap 概览。

(对应文件为 Figure+Table/SCI-genes-correlation-heatmap.pdf)

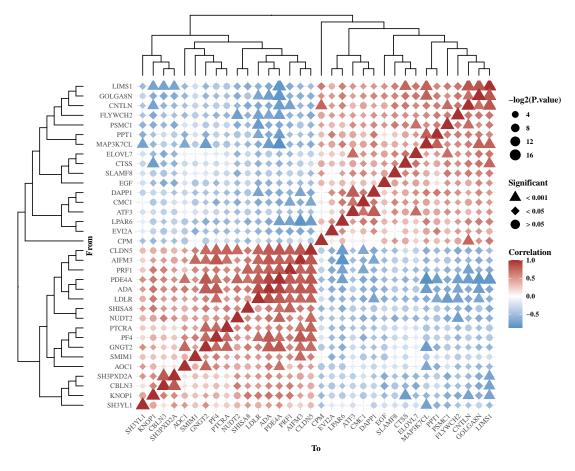


Figure 4: SCI genes correlation heatmap

Table 5 (下方表格) 为表格 SCI data significant genes of correlation 概览。

(对应文件为 Figure+Table/SCI-data-significant-genes-of-correlation.csv)

注: 表格共有 796 行 7 列, 以下预览的表格可能省略部分数据; 表格含有 34 个唯一'From'。

- 1. cor: 皮尔逊关联系数, 正关联或负关联。
- 2. pvalue: 显著性 P。
- 3. -log2(P.value): P 的对数转化。
- 4. significant: 显著性。
- 5. sign: 人为赋予的符号,参考 significant。

Table 5: SCI data significant genes of correlation

From	То	cor	pvalue	-log2	signi	sign
ADA	ADA	1	0	16.60	< 0.001	**
PPT1	ADA	-0.66	0.0022	8.828	< 0.05	*
LDLR	ADA	0.86	0	16.60	< 0.001	**
EVI2A	ADA	-0.63	0.0036	8.117	< 0.05	*

From	То	cor	pvalue	-log2	signi	sign
CPM	ADA	-0.46	0.0452	4.467	< 0.05	*
KNOP1	ADA	0.69	0.0012	9.702	< 0.05	*
AIFM3	ADA	0.82	0	16.60	< 0.001	**
CBLN3	ADA	0.59	0.0077	7.020	< 0.05	*
PRF1	ADA	0.77	1e-04	13.28	< 0.001	**
AOC1	ADA	0.66	0.0019	9.039	< 0.05	*
PDE4A	ADA	0.91	0	16.60	< 0.001	**
CNTLN	ADA	-0.65	0.0027	8.532	< 0.05	*
FLYWCH2	ADA	-0.7	8e-04	10.28	< 0.001	**
SH3YL1	ADA	0.5	0.0301	5.054	< 0.05	*
NUDT2	ADA	0.69	0.0011	9.828	< 0.05	*

6.3.3 NP 的 coDEGs 的关联性分析

Figure 5 (下方图) 为图 NP genes correlation heatmap 概览。

(对应文件为 Figure+Table/NP-genes-correlation-heatmap.pdf)

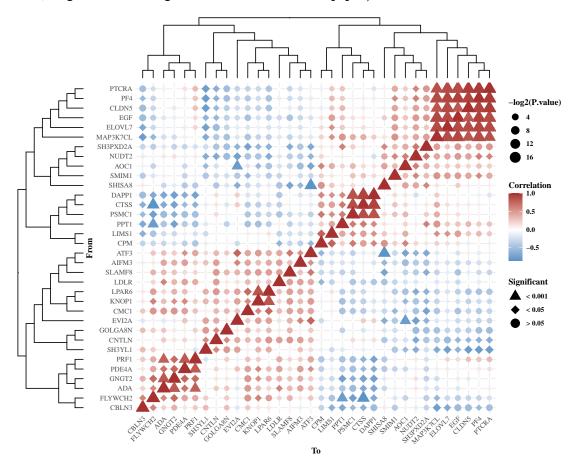


Figure 5: NP genes correlation heatmap

Table 6 (下方表格) 为表格 NP data significant genes of correlation 概览。

(对应文件为 Figure+Table/NP-data-significant-genes-of-correlation.csv)

注: 表格共有 262 行 7 列, 以下预览的表格可能省略部分数据; 表格含有 34 个唯一'From'。

- 1. cor: 皮尔逊关联系数, 正关联或负关联。
- 2. pvalue: 显著性 P。
- 3. -log2(P.value): P 的对数转化。
- 4. significant: 显著性。
- 5. sign: 人为赋予的符号,参考 significant。

Table 6: NP data significant genes of correlation

From	То	cor	pvalue	-log2	signi	sign
SMIM1	SMIM1	1	0	16.60	< 0.001	**
CMC1	SMIM1	-0.56	0.0377	4.729	< 0.05	*
PF4	SMIM1	0.58	0.0302	5.049	< 0.05	*
NUDT2	SMIM1	0.59	0.0264	5.243	< 0.05	*
PPT1	PPT1	1	0	16.60	< 0.001	**
CTSS	PPT1	0.66	0.01	6.643	< 0.05	*
DAPP1	PPT1	0.58	0.028	5.158	< 0.05	*
SH3PXD2A	PPT1	0.62	0.0173	5.853	< 0.05	*
CBLN3	PPT1	-0.68	0.0069	7.179	< 0.05	*
PSMC1	PPT1	0.64	0.0135	6.210	< 0.05	*
FLYWCH2	PPT1	-0.78	9e-04	10.11	< 0.001	**
GNGT2	PPT1	-0.74	0.0026	8.587	< 0.05	*
PPT1	CTSS	0.66	0.01	6.643	< 0.05	*
CTSS	CTSS	1	0	16.60	< 0.001	**
DAPP1	CTSS	0.92	0	16.60	< 0.001	**

6.3.4 SCI 和 NP 数据集共同显著关联的基因集 sigCoDEGs

6.4 重复经颅磁刺激治疗 (repeat transcranial magnetic stimulation, rTMS) (Rat)

6.4.1 元数据

Table 7 (下方表格) 为表格 rTMS used sample metadata 概览。

(对应文件为 Figure+Table/rTMS-used-sample-metadata.csv)

注: 表格共有 56 行 11 列,以下预览的表格可能省略部分数据;表格含有 12 个唯一'group'。

sample: 样品名称
 group: 分组名称

Table 7: RTMS used sample metadata

sample	group	title	age.ch1	cogni	post	Sex.ch1	strai	tissu9	tissu10
GSM71	Y.H.Sham	Y_Sha	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.Sham	Y_Sha	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.Sham	Y_Sha	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.Sham	Y_Sha	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.iTBS	Y_iTB	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.iTBS	Y_iTB	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.iTBS	Y_iTB	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	Y.H.iTBS	Y_iTB	Young	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_Sh	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_Sh	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_Sh	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_Sh	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_iT	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_iT	Aged;	unimp	48	Male	Long	Hippo	Brain
GSM71	AU.H	AU_iT	Aged;	unimp	48	Male	Long	Hippo	Brain

6.4.2 差异分析

Figure 6 (下方图) 为图 rTMS All DEGs of contrasts 概览。

(对应文件为 Figure+Table/rTMS-All-DEGs-of-contrasts.pdf)

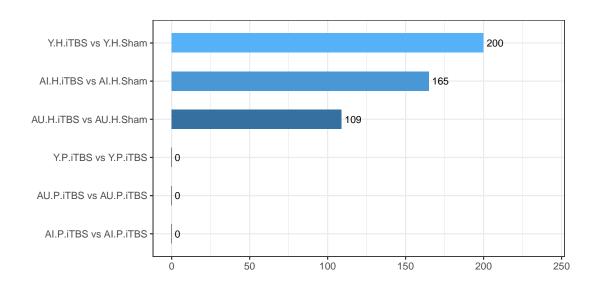


Figure 6: RTMS All DEGs of contrasts

'RTMS data DEGs' 数据已全部提供。

(对应文件为 Figure+Table/rTMS-data-DEGs)

注:文件夹 Figure+Table/rTMS-data-DEGs 共包含 6 个文件。

- 1. 1_AU.H.iTBS AU.H.Sham.csv
- 2. 2_AU.P.iTBS AU.P.iTBS.csv
- 3. 3_AI.H.iTBS AI.H.Sham.csv
- 4. 4_AI.P.iTBS AI.P.iTBS.csv
- 5. 5_Y.H.iTBS Y.H.Sham.csv
- 6. ...

6.5 rTMS 和 SCI、NP 的关联

6.5.1 rTMS 数据的差异基因与 sig.coDegs 的关联性

Figure 7 (下方图) 为图 NP sigCoDEGs with rTMS DEGs correlation heatmap 概览。

(对应文件为 Figure+Table/NP-sigCoDEGs-with-rTMS-DEGs-correlation-heatmap.pdf)

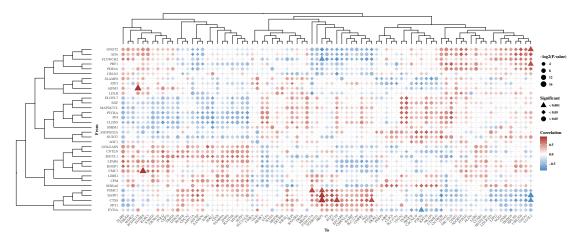


Figure 7: NP sigCoDEGs with rTMS DEGs correlation heatmap

Table 8 (下方表格) 为表格 NP sigCoDEGs with rTMS DEGs significant correlation 概览。

(对应文件为 Figure+Table/NP-sigCoDEGs-with-rTMS-DEGs-significant-correlation.csv)

注: 表格共有 297 行 7 列, 以下预览的表格可能省略部分数据; 表格含有 34 个唯一'From'。

1. cor: 皮尔逊关联系数, 正关联或负关联。

2. pvalue: 显著性 P。

3. -log2(P.value): P 的对数转化。

4. significant: 显著性。

5. sign: 人为赋予的符号,参考 significant。

Table 8: NP sigCoDEGs with rTMS DEGs significant correlation

From	То	cor	pvalue	-log2	signi	sign
CMC1	UBXN10	0.58	0.0311	5.006	< 0.05	*
SH3PXD2A	UBXN10	-0.64	0.0142	6.137	< 0.05	*
CTSS	LDLRAP1	-0.61	0.0202	5.629	< 0.05	*
SHISA8	LDLRAP1	0.54	0.047	4.411	< 0.05	*
SH3YL1	ZFP69	0.55	0.0417	4.583	< 0.05	*
CPM	ZFP69	0.56	0.0381	4.714	< 0.05	*
LPAR6	ZFP69	0.54	0.0451	4.470	< 0.05	*
CLDN5	ZFP69	-0.57	0.0332	4.912	< 0.05	*
CMC1	FOXD2	0.58	0.0305	5.035	< 0.05	*
PRF1	FOXD2	0.6	0.0222	5.493	< 0.05	*
GNGT2	FOXD2	0.62	0.0186	5.748	< 0.05	*
PDE4A	FOXD2	0.58	0.0299	5.063	< 0.05	*
ADA	FOXD2	0.61	0.02	5.643	< 0.05	*
PPT1	PTGFR	0.59	0.0254	5.299	< 0.05	*

From	То	cor	pvalue	-log2	signi	sign
CTSS	PTGFR	0.79	7e-04	10.48	< 0.001	**

Figure 8 (下方图) 为图 SCI sigCoDEGs with rTMS DEGs correlation heatmap 概览。

(对应文件为 Figure+Table/SCI-sigCoDEGs-with-rTMS-DEGs-correlation-heatmap.pdf)

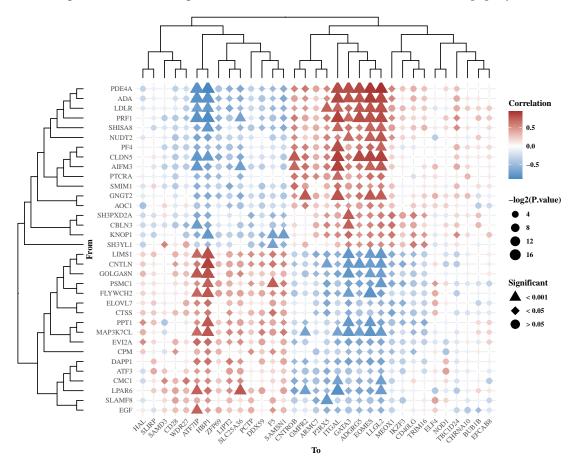


Figure 8: SCI sigCoDEGs with rTMS DEGs correlation heatmap

Table 9 (下方表格) 为表格 SCI sigCoDEGs with rTMS DEGs significant correlation 概览。

(对应文件为 Figure+Table/SCI-sigCoDEGs-with-rTMS-DEGs-significant-correlation.csv)

注: 表格共有 406 行 7 列, 以下预览的表格可能省略部分数据; 表格含有 34 个唯一'From'。

1. cor: 皮尔逊关联系数, 正关联或负关联。

2. pvalue: 显著性 P。

3. -log2(P.value): P 的对数转化。

4. significant: 显著性。

5. sign: 人为赋予的符号,参考 significant。

Table 9: SCI sigCoDEGs with rTMS DEGs significant correlation

From	То	cor	pvalue	-log2	signi	sign
PPT1	CD40LG	-0.54	0.0173	5.853	< 0.05	*
CPM	CD40LG	-0.52	0.0214	5.546	< 0.05	*
KNOP1	CD40LG	0.54	0.017	5.878	< 0.05	*
CNTLN	CD40LG	-0.49	0.0331	4.917	< 0.05	*
SH3YL1	CD40LG	0.62	0.0046	7.764	< 0.05	*
LIMS1	CD40LG	-0.5	0.028	5.158	< 0.05	*
${\rm MAP3K7CL}$	CD40LG	-0.52	0.0231	5.435	< 0.05	*
SH3PXD2A	CD40LG	0.61	0.0052	7.587	< 0.05	*
ADA	F5	-0.47	0.0425	4.556	< 0.05	*
LDLR	F5	-0.52	0.0228	5.454	< 0.05	*
CPM	F5	0.52	0.021	5.573	< 0.05	*
KNOP1	F5	-0.7	8e-04	10.28	< 0.001	**
PDE4A	F5	-0.5	0.0285	5.132	< 0.05	*
CNTLN	F5	0.63	0.0036	8.117	< 0.05	*
FLYWCH2	F5	0.52	0.0237	5.398	< 0.05	*

6.5.2 富集分析

Figure 9 (下方图) 为图 RTMS SCI NP correlated sci GO enrichment 概览。

(对应文件为 Figure+Table/RTMS-SCI-NP-correlated-sci-GO-enrichment.pdf)

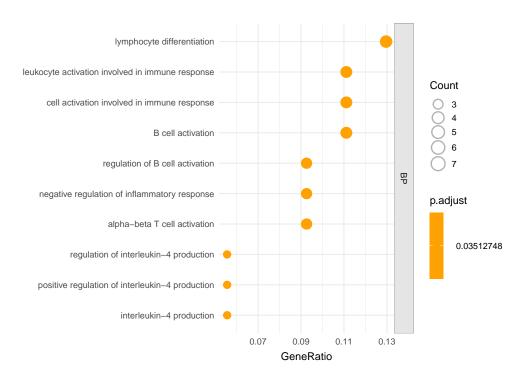


Figure 9: RTMS SCI NP correlated sci GO enrichment

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- 1. Wu, T. et al. ClusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation 2, (2021).
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- 4. Morrison, D. et al. Profiling immunological phenotypes in individuals during the first year after traumatic spinal cord injury: A longitudinal analysis. *Journal of Neurotrauma* 40, 2621–2637 (2023).