Analysis

Huang LiChuang of Wie-Biotech

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1   题目

转录组数据集结合差异分析与基因共表达分析筛选结直肠癌化疗与肌少症的关联通路

2   摘要

结直肠癌（colorectal cancer）的化疗（Chemotherapy）可能导致或加重肌少症（Sarcopenia），然而其内在机制尚不明朗。为了筛选共通的通路，本研究选取了 2 个 GEO 的结直肠癌细胞系数据集，2 个 TCGA 结肠癌或直肠癌数据集，1 个肌少症的 GEO 数据集，结合了差异分析、基因共表达分析、富集分析等方式，探究可能的内在机制。通路富集表明，自噬（Autophagy）和线粒体自噬（Mitophagy）是共同通路，与 Autophagy 和 Mitophagy 的共交集基因为 RAB7A, CALCOCO2, BNIP3, ATG9A。其中，BNIP3 在 Sarcopenia 的数据集和 TCGA 直肠数据集中同时差异表达（P < 0.05）。

PS：结果未找到与 KEAP1-NRF2 通路的关联。

3   前言

应用化疗治疗可刺激癌症细胞施放内源性危险信号，以诱导发生免疫反应[1]。然而，化疗过程可伴随副作用的发生，例如肌少症[2]。肌少症是一种在老年人中常见的，一种进行性、全身性骨骼肌疾病，涉及肌肉质量和功能的加速丧失，与跌倒、功能下降、虚弱和死亡等不良后果[3]。肌少症的发生发展涉及多种机制，诸如激素（IGF-1 和胰岛素等）的功能、线粒体功能过程中的细胞内机制等[4]。在恶性肿瘤的进展中，和相关的营养不良本身就会侵蚀肌肉质量，诱发肌少症，而化疗会发生类似的机制，并且会加重这个过程[2]。尽管已有文献报导恶性肿瘤、化疗、肌少症之间的相关性，然而其内在的分子机制尚不明朗。本研究从结直肠癌（CRC）出发，选用公共数据库的多个数据集，分别以 CRC 细胞系层面、临床样本层面筛选化疗的差异表达基因，结合肌少症的转录组数据的基因共表达分析，探究潜在的结直肠癌化疗与肌少症的共通通路。

4 研究设计流程图
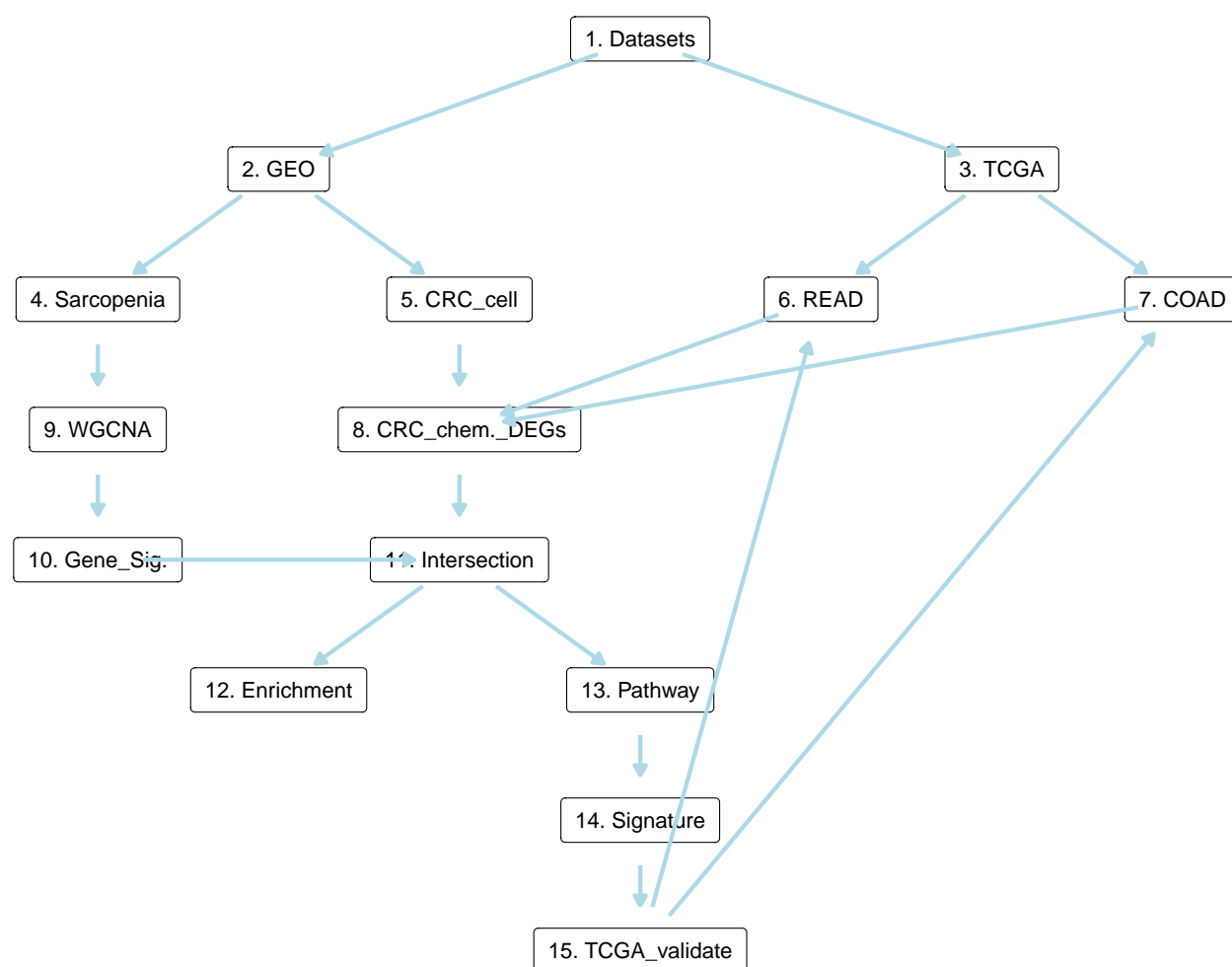
5 材料和方法

5.1 Methods

主要 R 包：

- GEOquery
- TCGAbiolinks
- edgeR
- limma
- clusterProfiler
- pathview
- …

5.2 meterials

数据集：

- GSE142340
- GSE153412
- TCGA-READ
- TCGA-COAD
- GSE167186

具体见 8

6 分析结果

6.1 结直肠癌细胞的特定药物化疗差异分析

为了探究化疗所致的癌症共性的生理或病理变化，此处首先从细胞层面探究化疗导致的差异表达基因。两个来源于 GEO 的数据集被采用，分别是 GSE142340 (Geo1) 和 GSE153412 (Geo2)。Geo1 包含六种 CRC (colorectal cancer) 细胞系的化疗的对比分析，涉及药物 Regorafenib、Selumetinib、Vemurafenib、Vatalanib、AZD-4547、GDC-0994 混合应用（Tab. 1）[5]。Geo2 包含三种 CRC 细胞系的化疗对比分析，涉及药物 5-fluorouracil (和 Uracil 对照)（Tab. 2）[6]。将数据预处理、经差异分析后，Geo1 和 Geo2 的各组差异表达基因（p-value < 0.05, $|\log2(\text{FC})| > 0.3$）的 UpSet 图分别见 Fig. 1a 和 b。在 Fig. 1b 中，相比于药物敏感型，耐受型几乎不发生基因转录水平的变化。分别取 Geo1 和 Geo2（不包括药物耐受组的差异基因）的共交集基因，以供后续分析。

Figure 1为图 MAIN DEGs obtained from two GEO datasets about colorectal chemotherapy 概览。

(对应文件为 ./Figure+Table/fig1.pdf)

a

b
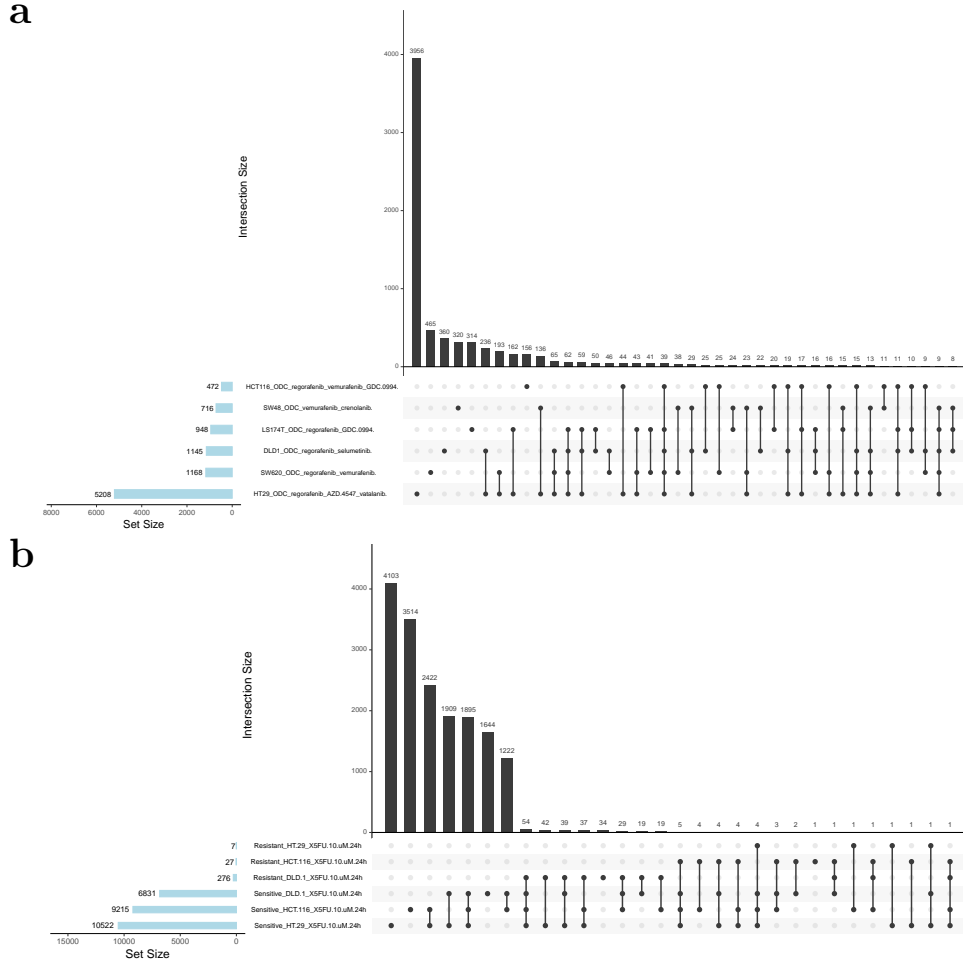
Figure 1: MAIN DEGs obtained from two GEO datasets about colorectal chemotherapy

6.2 TCGA 临床样本的泛型化疗差异分析

为了从个体水平探究化疗对于癌症转录水平上的影响，从 TCGA 数据库获取结肠癌（READ）和直肠癌（ROAD）转录组数据以及相应的临床数据，根据是否药物化疗进行分组。TCGA-READ 共包含 135 个患者的数据（Tab. 3），其化疗分布占比见 Fig. 2；TCGA-COAD 共包含 388 个患者的数据（Tab. 4），其化疗分布占比见 Fig. 2c。分别将两批数据去除未记录是否化疗的样本后，标准化数据后，以未化疗组和化疗组进行差异分析（Fig. 2b 和 d），供后续使用。需要注意的是，该 TCGA 的临床数据未记录患者的用药种类、周期等信息，相对于细胞层面的差异分析，更具不稳定性，但也更贴近实际化疗情形。筛选所得的差异基因（p-value < 0.05, $|\log2(FC)| > 0.3$）数见 Fig. 4a。

Figure 2为图 MAIN DEGs obtained from two TCGA project datasets about colorectal chemotherapy 概览。

（对应文件为 ./Figure+Table/fig2.pdf）
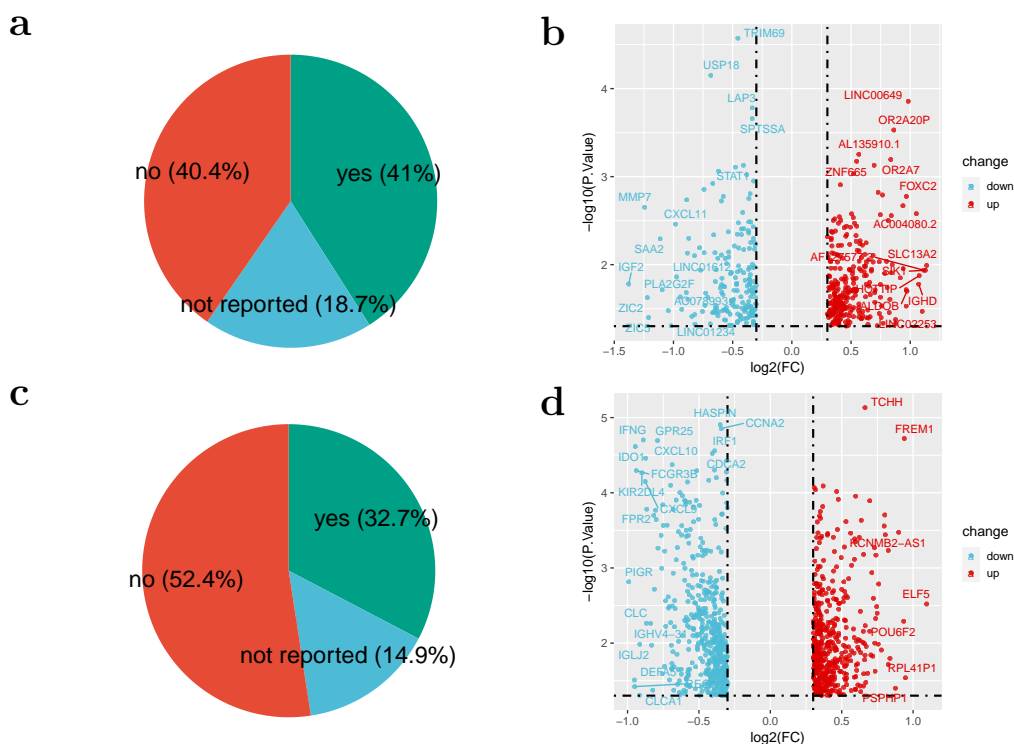
Figure 2: MAIN DEGs obtained from two TCGA project datasets about colorectal chemotherapy

6.3　肌少症的基因共表达分析

肌少症的数据集来源于 GEO 的 GSE167186。选取肌少症患者组和对照组的样本（Old Sarcopenia, Old Healthy），以筛选肌少症的潜在标志基因 (Tab. 5)。该数据集包含肌少症指标的临床数据，故采用 WGCNA[7] 替代差异分析，以避免多重比较的 p 值矫正问题。选择 Soft Threshold 为 2（Fig. 3a），建立基因共表达模块（Fig. 3b）。随后，结合临床数据筛选显著基因模块（Fig. 3d）。临床数据显著关联的基因集（GS，gene significant）和显著的基因模块关系（MM，module membership）的交集（Fig. 3c），共有 1779 个基因。

Figure 3为图 MAIN significant genes obtained by WGCNA analysis of GEO Sarcopenia dataset 概览。

(对应文件为 ./Figure+Table/fig3.pdf)
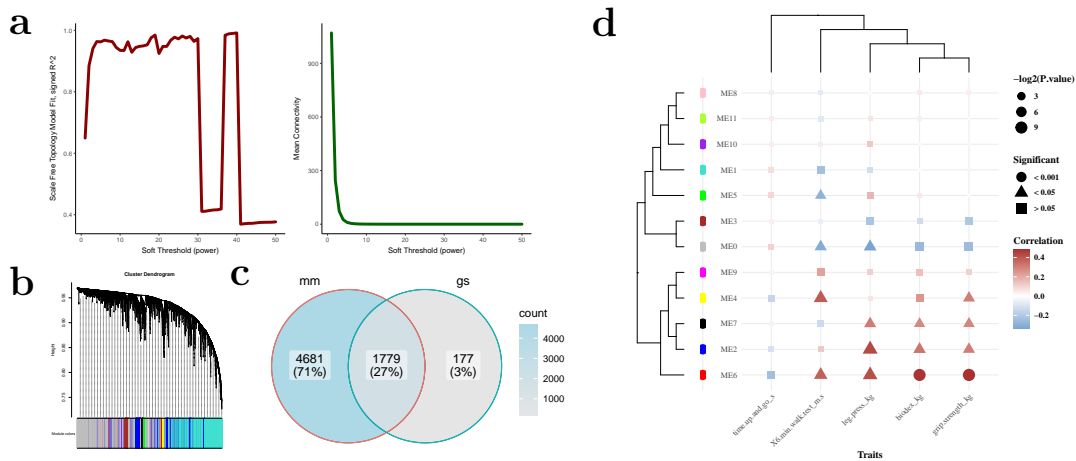
Figure 3: MAIN significant genes obtained by WGCNA analysis of GEO Sarcopenia dataset

6.4 结直肠癌与肌少症的综合分析

以转录组数据库串连肌少症（Sarcopenia）、结直肠癌（colorectal cancer）、化疗（Chemotherapy）筛选共同的通路，取上述结直肠癌差异基因（6.1 和 6.2）（Fig. 4a）的合集，与肌少症的潜在标志基因取交集（6.3）（Fig. 4c）。共有 184 个交集基因。KEGG 富集分析表明（Fig. 4d），自噬（Autophagy）通路为首要富集的通路；此外，线粒体自噬（Mitophagy）也是显著的通路。GO 富集分析同样揭示了（Fig. 4b）线粒体相关的通路，如，BP: 'mitochondrial protein processing', CC: 'mitochondrial inner membrane', CC: 'autophagosome' 等。自噬通路（Autophagy）（Fig. 4e）与 Hypoxia、Low energy、ROS 等肿瘤微环境因素相关。事实上，已有文献综述了线粒体（线粒体失调、氧化应激等）、衰老、肿瘤、肌少症[8-10] 之间的关联。

PS: The Cellular Component (CC), the Molecular Function (MF) and the Biological Process (BP).

Figure 4为图 MAIN The intersected genes enriched in mitochondrial and autophagy related pathway 概览。

（对应文件为 ./Figure+Table/fig4.pdf）
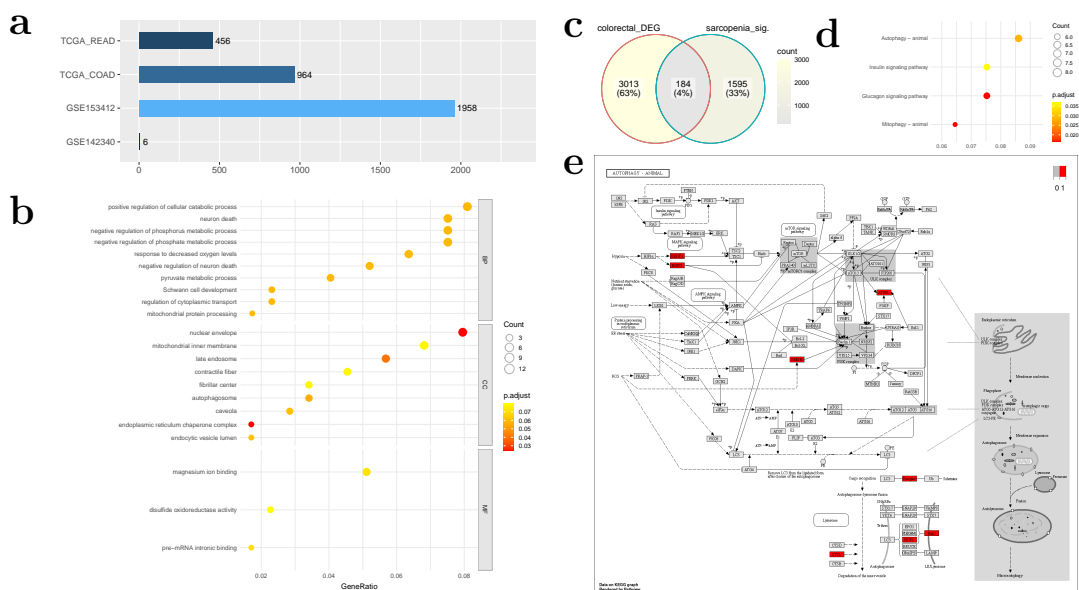
Figure 4: MAIN The intersected genes enriched in mitochondrial and autophagy related pathway

6.5 线粒体自噬基因的表达

聚焦于线粒体自噬通路（Mitophagy）的 6 个交集基因（Fig. 5a）。这 6 个基因与自噬通路有 4 个交集基因，分别为 RAB7A, CALCOCO2, BNIP3, ATG9A。在上述分析中，我们以多重数据的差异分析的合集，结合 WGCNA 分析的方式取得基因集（Fig. 4c），接下来，为了验证 Mitophagy 的 6 个基因的显著性，重新回到对应的数据集检查。在 Sarcopenia 的数据集中，BNIP3, JUN 为差异表达的基因（Fig. 5b）。随后，可以在 TCGA-COAD 数据集中可以发现 BNIP3 的差异表达（Fig. 5d）；然而，BNIP3 在 TCGA-READ 数据集为非差异表达基因（Fig. 5c）。显然，该 6 个基因的其余基因是来自于纯细胞系的转录组数据筛选，与复杂的临床样本有所不同。上述分析表明，基因 BNIP3 在直肠癌的化疗治疗中，可能与肌少症的发生发展机制关联，并且与线粒体自噬通路相关。然而，BNIP3 与肌少症的关联机制是否存在于结肠癌或者其它癌症的化疗过程中，需要进一步验证。

Figure 5为图 MAIN The expression of mitophgy related genes in colorectal cancer or sarcopenia 概览。

（对应文件为 ./Figure+Table/fig5.pdf）
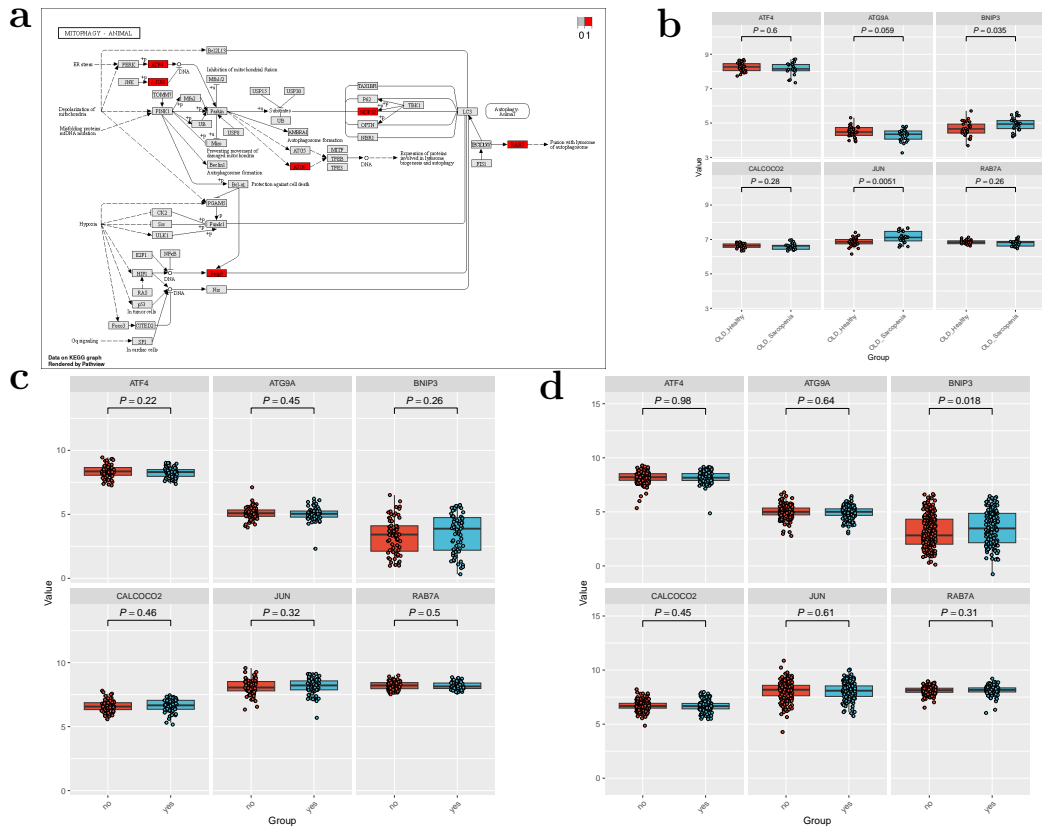
Figure 5: MAIN The expression of mitophgy related genes in colorectal cancer or sarcopenia

7　结论

结直肠癌（colorectal cancer）的化疗（Chemotherapy）与肌少症（Sarcopenia）的关联通路为自噬（Autophagy）和线粒体自噬（Mitophagy）。Autophagy 和 Mitophagy 的共交集基因为 RAB7A, CALCOCO2, BNIP3, ATG9A。其中，BNIP3 在直肠癌的化疗治疗和肌少症患者中的表达均显著升高。

8　附：分析流程

8.1　相关文献

- Skeletal muscle-specific Keap1 disruption modulates fatty acid utilization and enhances exercise capacity in female mice[11]
- Mitochondrial dysfunction and oxidative stress in aging and cancer[8]
- Sarcopenia in the Older Adult With Cancer[9]
- Danger signals: Chemotherapy enhancers?[1]
- Current Targeted Therapy for Metastatic Colorectal Cancer[12]

- The role of aging in cancer[10]

8.2 GEO 结直肠癌（细胞样本）

8.2.1 GSE142340: six CRC cell lines treated with CTRL and optimized drug combinations (ODCs)

- RNA sequencing was conducted for six CRC cell lines treated with CTRL and optimized drug combinations (ODCs), providing samples in duplicate
  - GSE142340

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data_processing :

Illumina Casava2.2 software used for basecalling.

data_processing.1 :

Sequenced reads were mapped to rn6 whole genome using STAR v2.5.3a with default parameters

data_processing.2 :

Raw counts are produced by htseq-count (HTSeq v.0.9.1)

data_processing.3 :

Normalization and differential expression analysis were performed with edgeR v.3.24.3

data_processing.4 :

Genome_build: hg38

data_processing.5 :

Supplementary_files_format_and_content: tab-delimited text files include rawcount values for each Sample

data_processing.6 :

Supplementary_files_format_and_content: tab-delimited text files include normalized expression values(cpm = count per millions) for each Sample

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Table 1为表格 metadata of GSE142340 概览。

（对应文件为 Figure+Table/metadata-of-GSE142340.csv）

Table 1: Metadata of GSE142340

rownames	group	lib.size	norm....	sample	cell....	treat...
DLDCTR2	DLD1_...	22435...	1.002...	DLDCTR2	DLD1	CTRL
DLDCTRL1	DLD1_...	19842...	0.982...	DLDCTRL1	DLD1	CTRL
DLDODC1	DLD1_...	22132...	0.979...	DLDODC1	DLD1	ODC.....
DLDODC2	DLD1_...	21319...	1.007...	DLDODC2	DLD1	ODC.....
HCTCTRL1	HCT11...	21546...	0.980...	HCTCTRL1	HCT116	CTRL
HCTCTRL2	HCT11...	23262...	0.972...	HCTCTRL2	HCT116	CTRL
HCTODC1	HCT11...	24506...	0.995...	HCTODC1	HCT116	ODC.....
HCTODC2	HCT11...	22573...	0.991...	HCTODC2	HCT116	ODC.....
HTCTRL1	HT29_...	21553...	0.930...	HTCTRL1	HT29	CTRL
HTCTRL2	HT29_...	22492...	0.953...	HTCTRL2	HT29	CTRL
HTODC1	HT29_...	16252...	0.819...	HTODC1	HT29	ODC.....
HTODC2	HT29_...	20979...	0.964...	HTODC2	HT29	ODC.....
LSCTRL1	LS174...	22752...	0.986...	LSCTRL1	LS174T	CTRL
LSCTRL2	LS174...	21163...	1.014...	LSCTRL2	LS174T	CTRL
LSODC1	LS174...	21870...	0.979...	LSODC1	LS174T	ODC.....
...	...	...	...	...	...	...

Figure 6为图 DEGs in different cell types with chemotherapy or not GSE142340 概览。

(对应文件为 Figure+Table/DEGs-in-different-cell-types-with-chemotherapy-or-not-GSE142340.pdf)
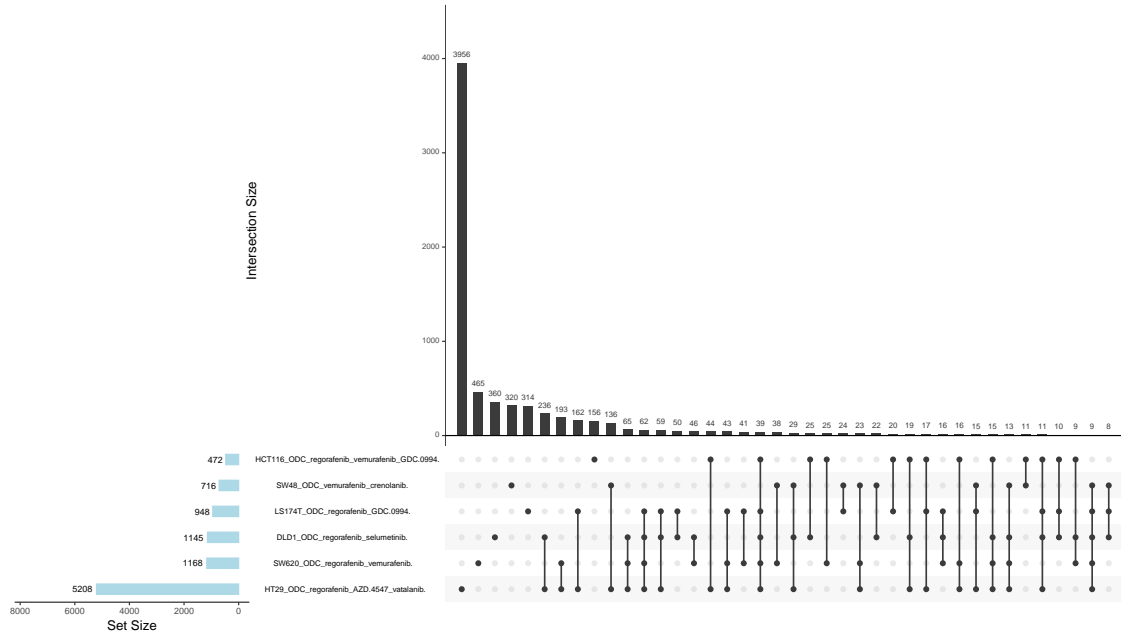
Figure 6: DEGs in different cell types with chemotherapy or not GSE142340

8.2.2 GSE153412: radio-chemoresistance in colorectal cancer cell lines

- RNAseq analysis of radio-chemoresistance in colorectal cancer cell lines
  - GSE153412

data_processing :

FASTQ files were generated using bcl2fastq version v2.20.0.422

data_processing.1 :

FASTQ files were filtered using Trimmomatic 0.36 (PE ILLUMINA-CLIP:adapters.fa:2:30:10 LEADING:5 TRAILING:5 MINLEN:45).

data_processing.2 :

The quality was assessed with FastQC 0.11.8.

data_processing.3 :

The transcriptome was generated with gffread, using the GRCh38.p13 genome and the latest Ensembl annotation (Homo sapiens version 98).

data_processing.4 :

Complete read pairs were aligned and quantified on a human transcriptome using Kallisto 0.44.0 (index built with –kmer-size=31).

data_processing.5 :

Genome_build: GRCh38.p13

data_processing.6 :

Supplementary_files_format_and_content: Kallisto output tsv file with transcripts abundance (raw counts and tpm).

data_processing.7 :

Supplementary_files_format_and_content: Matrix table with raw gene counts for every gene and every sample

data_processing.8 :

Supplementary_files_format_and_content: Matrix table with tpm values for every gene and every sample

Table 2为表格 metadata of GSE153412 概览。

(对应文件为 Figure+Table/metadata-of-GSE153412.csv)

> 注: 表格共有 54 行 8 列, 以下预览的表格可能省略部分数据; 表格含有 18 个唯一 'group'。

Table 2: Metadata of GSE153412

rownames	group	lib.size	norm....	sample	X5.fu....	cell....	treat...
DSU1	Sensi...	52675...	0.940...	DSU1	Sensi...	DLD.1	Uraci...
DSU2	Sensi...	20781...	0.951...	DSU2	Sensi...	DLD.1	Uraci...
DSU3	Sensi...	54236...	0.945...	DSU3	Sensi...	DLD.1	Uraci...
DS51	Sensi...	89157...	1.029...	DS51	Sensi...	DLD.1	X5FU....
DS52	Sensi...	61094...	1.016...	DS52	Sensi...	DLD.1	X5FU....
DS53	Sensi...	49329...	1.014...	DS53	Sensi...	DLD.1	X5FU....
DS5I1	Sensi...	68251...	1.022...	DS5I1	Sensi...	DLD.1	X5FU....
DS5I2	Sensi...	40788...	1.047...	DS5I2	Sensi...	DLD.1	X5FU....
DS5I3	Sensi...	58964...	1.035...	DS5I3	Sensi...	DLD.1	X5FU....
X116SU1	Sensi...	50504...	0.973...	X116SU1	Sensi...	HCT.116	Uraci...
X116SU2	Sensi...	52524...	1.004...	X116SU2	Sensi...	HCT.116	Uraci...
X116SU3	Sensi...	49999...	0.981...	X116SU3	Sensi...	HCT.116	Uraci...
X116S51	Sensi...	55051...	1.065...	X116S51	Sensi...	HCT.116	X5FU....
X116S52	Sensi...	81542...	1.033...	X116S52	Sensi...	HCT.116	X5FU....
X116S53	Sensi...	57667...	1.043...	X116S53	Sensi...	HCT.116	X5FU....
...	...	...	...	...	...	...	...

Figure 7为图 DEGs in different cell types with chemotherapy or not GSE153412 概览。

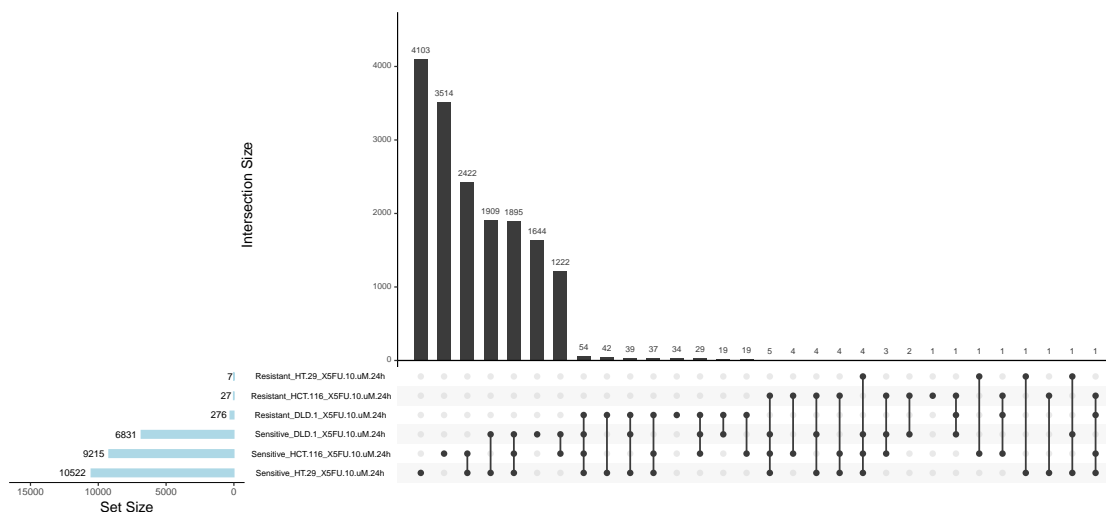**(对应文件为 Figure+Table/DEGs-in-different-cell-types-with-chemotherapy-or-not-GSE153412.pdf)**

Figure 7: DEGs in different cell types with chemotherapy or not GSE153412

8.3 TCGA 结肠癌（TCGA-READ）

Table 3为表格 READ clinical data 概览。

(对应文件为 `Figure+Table/READ-clinical-data.xlsx`)

> 注：表格共有 135 行 113 列，以下预览的表格可能省略部分数据；表格含有 135 个唯一
> 'sample'。

Table 3: READ clinical data

group	lib.size	norm....	sample	barcode	patient	short......7	defin...	sampl......9	sampl......10	...
yes	60935...	1.250...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	52344...	0.910...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
no	48571...	1.031...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	50713...	0.857...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
yes	60107...	0.945...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
no	47905...	0.993...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	19924...	0.898...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	63999...	1.069...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	21927...	1.060...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	51616...	0.974...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	33491...	0.871...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
yes	33640...	1.124...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	38780...	1.124...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	41384...	1.106...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	53539...	1.104...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
...	...	...	...	...	...	...	...	...	...	...

统计是否化疗：

Figure 8为图 READ whether with chemotherapy 概览。

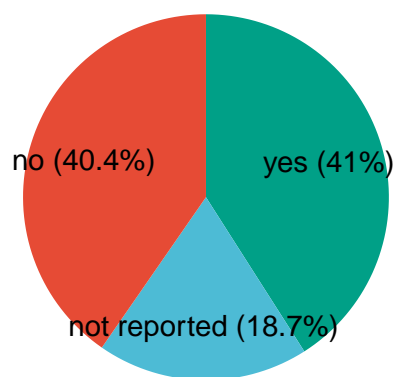**(对应文件为 `Figure+Table/READ-whether-with-chemotherapy.pdf`)**

Figure 8: READ whether with chemotherapy

Figure 9为图 READ difference expressed genes 概览。
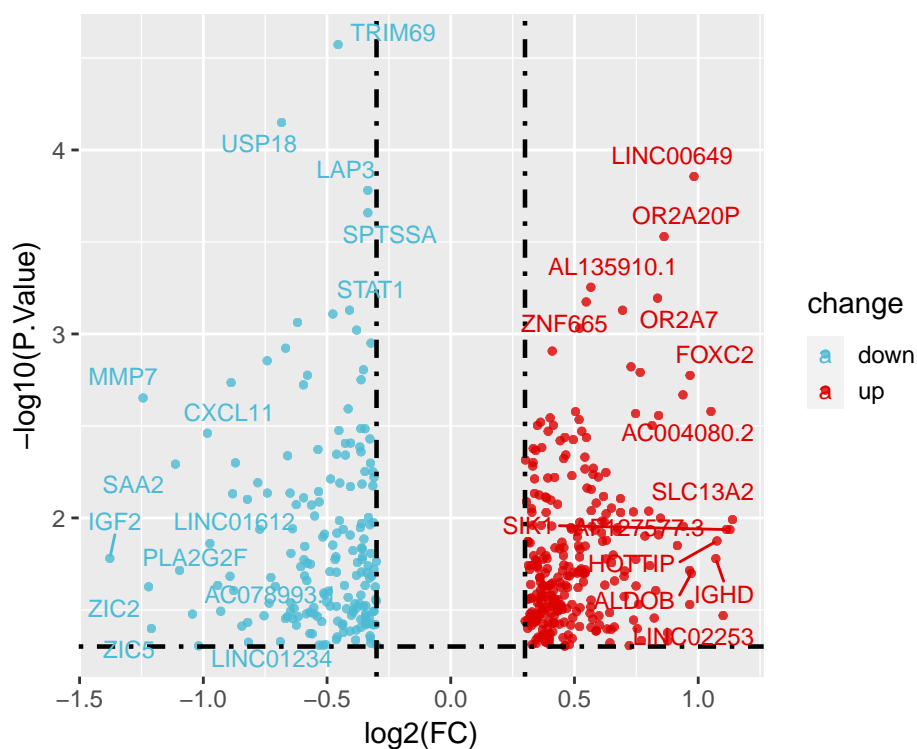
(对应文件为 Figure+Table/READ-difference-expressed-genes.pdf)

Figure 9: READ difference expressed genes

8.4 TCGA 直肠癌（TCGA-COAD）

Table 4为表格 COAD clinical data 概览。

(对应文件为 Figure+Table/COAD-clinical-data.xlsx)

> 注：表格共有 388 行 112 列，以下预览的表格可能省略部分数据；表格含有 388 个唯一
> 'sample'。

Table 4: COAD clinical data

group	lib.size	norm....	sample	barcode	patient	short......7	defin...	sampl......9	sampl......10	...
no	50725...	1.167...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	46391...	1.030...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	29081...	0.988...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	51150...	1.097...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	33677...	1.080...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	31679...	1.073...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	44720...	0.781...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
no	21922...	1.073...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	22321...	1.326...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
no	35916...	0.872...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
yes	19466...	0.969...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	30802...	1.147...	TCGA-...	TCGA-...	TCGA-...	TP	Prima...	TCGA-...	01	...
yes	52195...	0.909...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
no	40206...	0.831...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
no	46895...	0.751...	TCGA-...	TCGA-...	TCGA-...	NT	Solid...	TCGA-...	11	...
...	...	...	...	...	...	...	...	...	...	...

统计是否化疗：

Figure 10为图 COAD whether with chemotherapy 概览。

(对应文件为 `Figure+Table/COAD-whether-with-chemotherapy.pdf`)
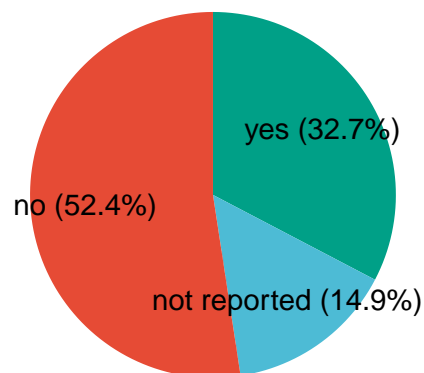

Figure 10: COAD whether with chemotherapy

使用 Limma 计算差异表达基因。

Figure 11为图 COAD difference expressed genes 概览。

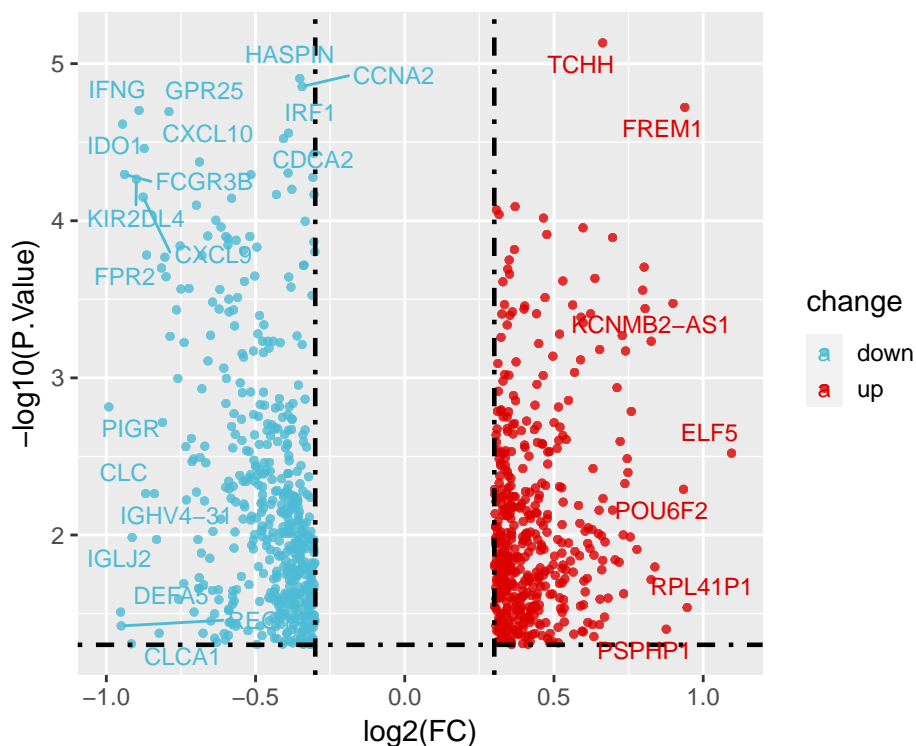**(对应文件为 Figure+Table/COAD-difference-expressed-genes.pdf)**

Figure 11: COAD difference expressed genes

8.5 GEO 肌少症

8.5.1 GSE167186: transcriptome profiling on lower limb muscle biopsies from 72 young, old and sarcopenic subjects

- We performed transcriptome profiling on lower limb muscle biopsies from 72 young, old and sarcopenic subjects using bulk RNA-seq (N = 72) and single-nuclei RNA-seq (N = 17).
  - GSE167186

8.5.1.1 edgeR 对数据进行标准化处理。

Figure 12为图 filtering of Sarcopenia datasets 概览。

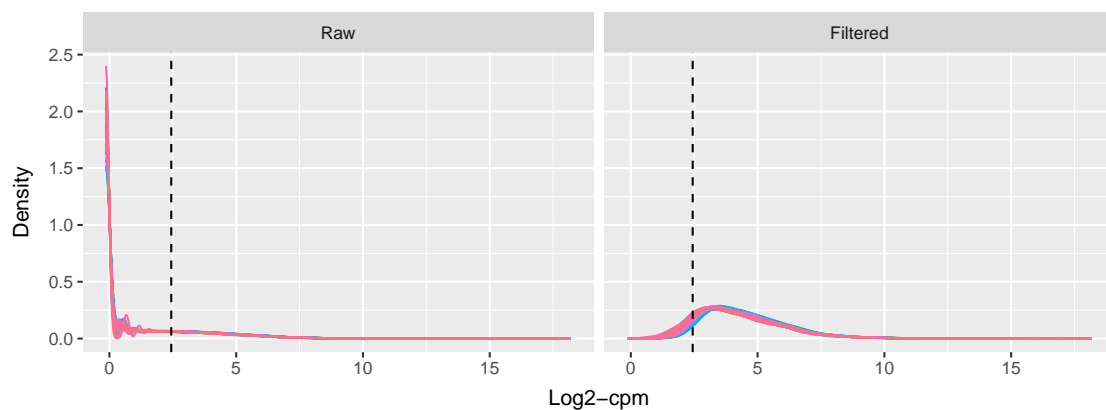**(对应文件为 Figure+Table/filtering-of-Sarcopenia-datasets.pdf)**

Figure 12: Filtering of Sarcopenia datasets

Figure 13为图 nomalization of Sarcopenia datasets 概览。

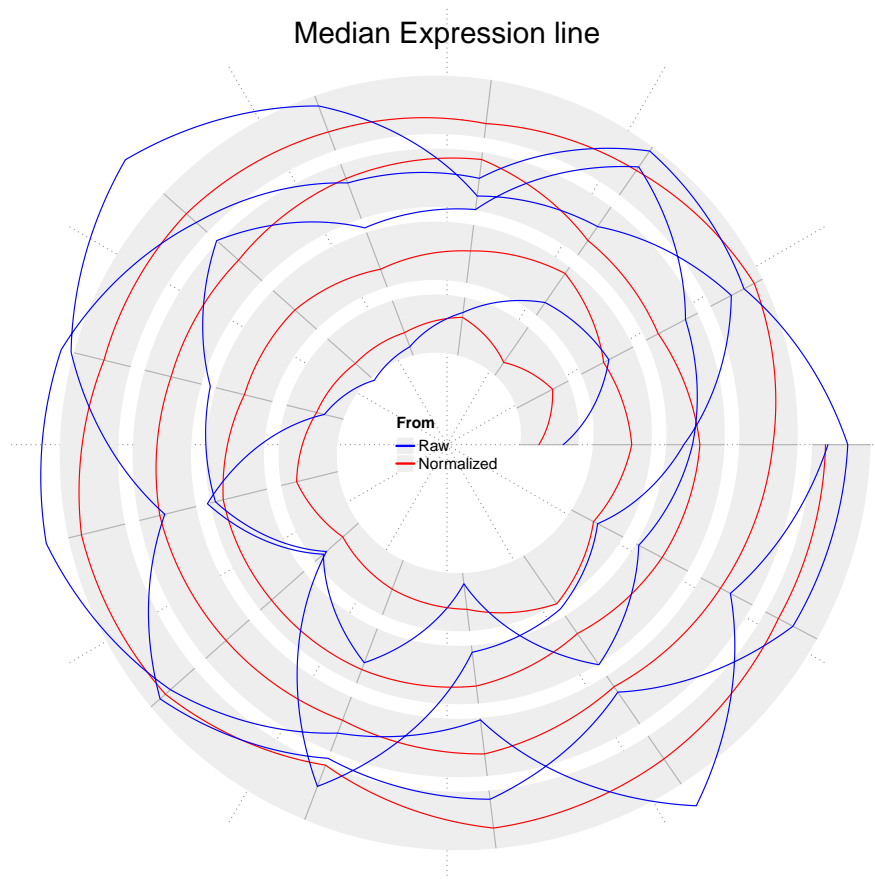**（对应文件为 `Figure+Table/nomalization-of-Sarcopenia-datasets.pdf`）**

Median Expression line

Figure 13: Nomalization of Sarcopenia datasets

8.5.1.2　WGCNA　Table 5为表格 metadata of samples used in GEO Sarcopenia data 概览。

（对应文件为 `Figure+Table/metadata-of-samples-used-in-GEO-Sarcopenia-data.csv`）

注：表格共有 53 行 16 列，以下预览的表格可能省略部分数据；表格含有 53 个唯一 'sample'。

Table 5: Metadata of samples used in GEO Sarcopenia data

rownames	group	lib.size	norm....	sample	X6.mi...	age.ch1	biode...	grip....	group...	leg.p...	...
X_10	OLD_S...	23299...	1.064...	X_10	1.010...	68	166.2	41.8	Sarco...	130.5	...
X_11	OLD_H...	24242...	1.078...	X_11	1.088...	87	136.4	24.2	Old H...	129.55	...
X_13	OLD_S...	17915...	0.968...	X_13	1.425...	83	143.1	40	Sarco...	127.27	...
X_14	OLD_H...	22048...	0.932...	X_14	0.918...	77	222.1	35.5	Old H...	229.5	...
X_15	OLD_H...	21527...	0.916...	X_15	NA	82	127.9	35.1	Old H...	94.5	...
X_16	OLD_H...	15754...	0.949...	X_16	NA	73	202.7	49.1	Old H...	145.5	...
X_17	OLD_S...	23226...	1.116...	X_17	0.886...	77	171.7	45.5	Sarco...	87.5	...
X_18	OLD_H...	22781...	0.998...	X_18	1.086...	64	203.9	42.7	Old H...	112.5	...
X_19	OLD_S...	21202...	1.092...	X_19	1.041...	77	143.8	33.7	Sarco...	112.5	...
X_1	OLD_S...	25372...	0.994...	X_1	NA	67	148.7	41.3	Sarco...	NA	...
X_20	OLD_S...	21469...	1.085...	X_20	1.136...	78	152.3	54.7	Sarco...	148.5	...
X_21	OLD_S...	23263...	1.041...	X_21	0.970...	83	100.6	25.8	Sarco...	67.5	...
X_22	OLD_H...	22763...	1.059...	X_22	1.025...	78	207.2	45.1	Old H...	139.5	...
X_23	OLD_H...	26356...	1.114...	X_23	1	76	236.8	42.4	Old H...	112.5	...
X_24	OLD_H...	30075...	1.185...	X_24	0.745...	65	120.8	23.6	Old H...	103.5	...
...	...	...	...	...	...	...	...	...	...	...	...

Figure 14为图 whether with Sarcopenia 概览。

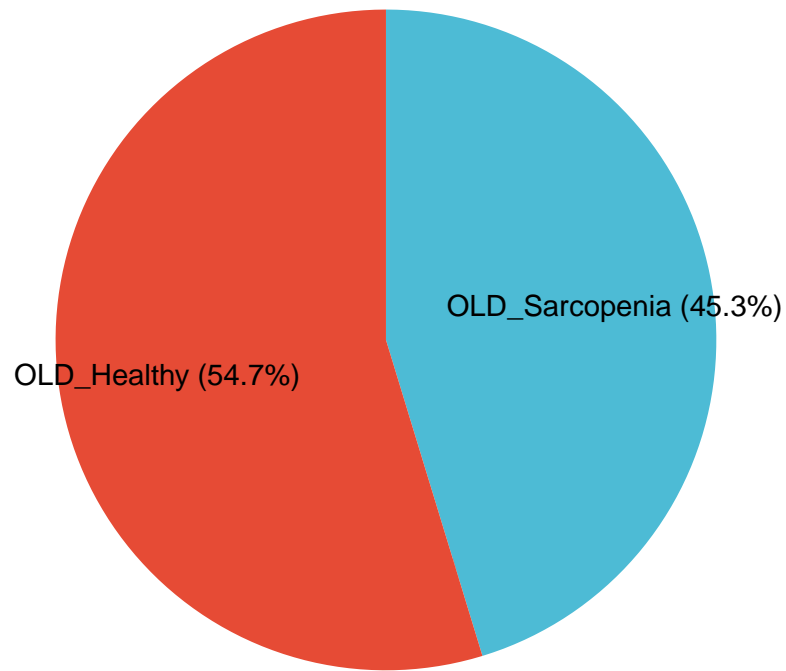**(对应文件为 Figure+Table/whether-with-Sarcopenia.pdf)**

Figure 14: Whether with Sarcopenia

data_processing :

Bulk RNA-seq was aligned using the STAR software, and counted using featureCounts.

data_processing.1 :

Single-nuclei RNA-seq was aligned using the CellRanger software (10x).

data_processing.2 :

Genome_build: Homo_sapiens.GRCh38

data_processing.3 :

Supplementary_files_format_and_content: counts.csv: Counts data for bulk RNA-seq.

data_processing.4 :

Supplementary_files_format_and_content: HM*.csv: Counts for single-nuclei RNA-seq.

Figure 15为图 soft threshold 概览。

(对应文件为 Figure+Table/soft-threshold.pdf)
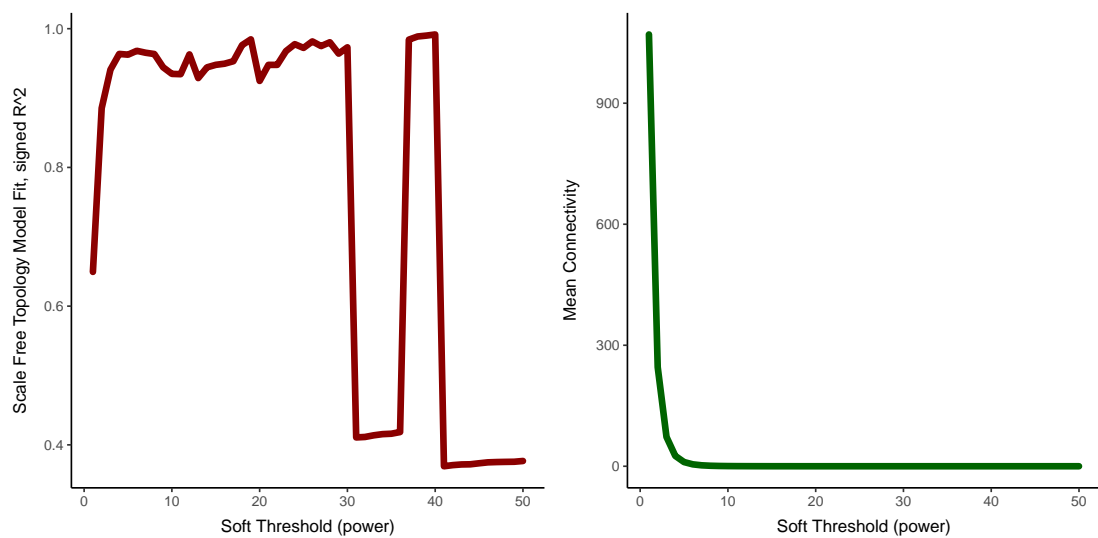
Figure 15: Soft threshold

Figure 16为图 clustering of gene modules 概览。

(对应文件为 Figure+Table/clustering-of-gene-modules.pdf)

Cluster Dendrogram
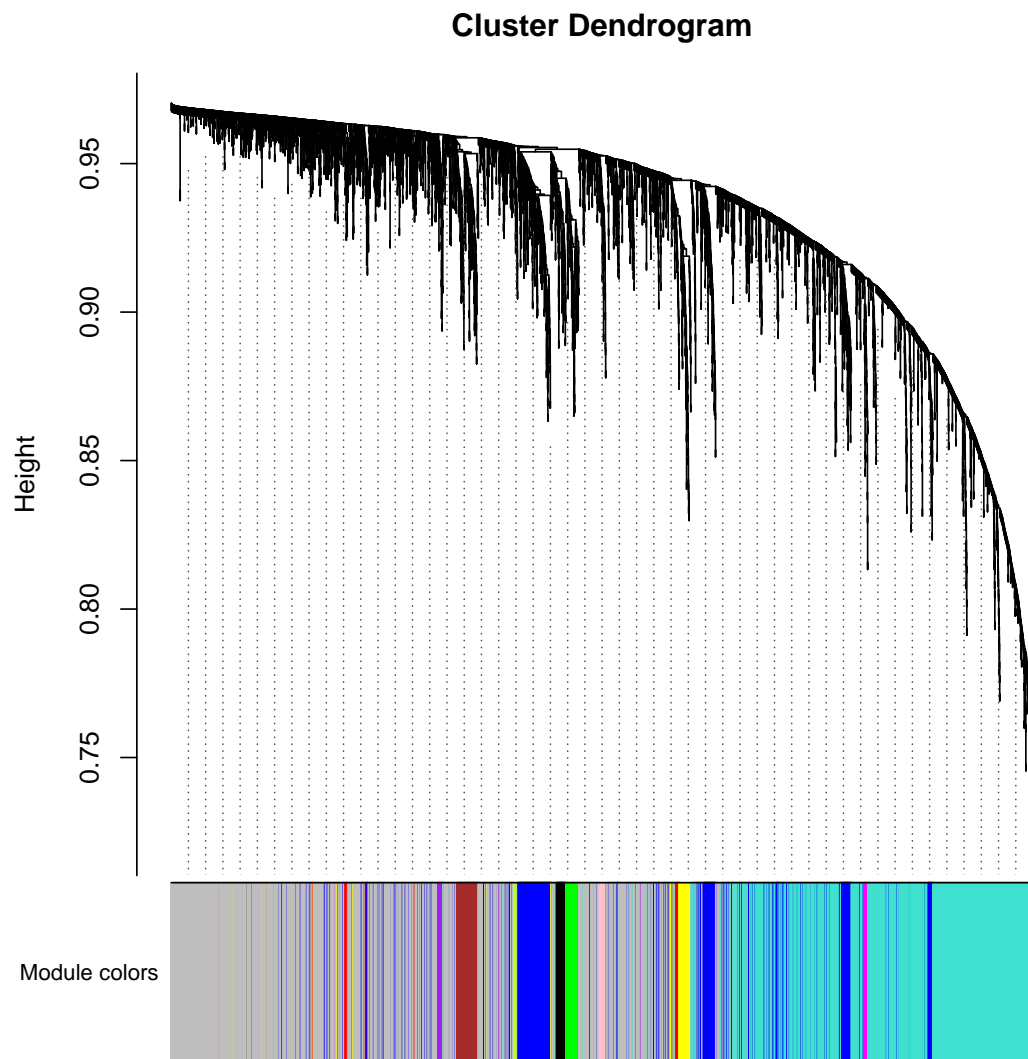
Figure 16: Clustering of gene modules

Figure 17为图 correlation of gene modules and traits data 概览。

(**对应文件为 Figure+Table/correlation-of-gene-modules-and-traits-data.pdf**)
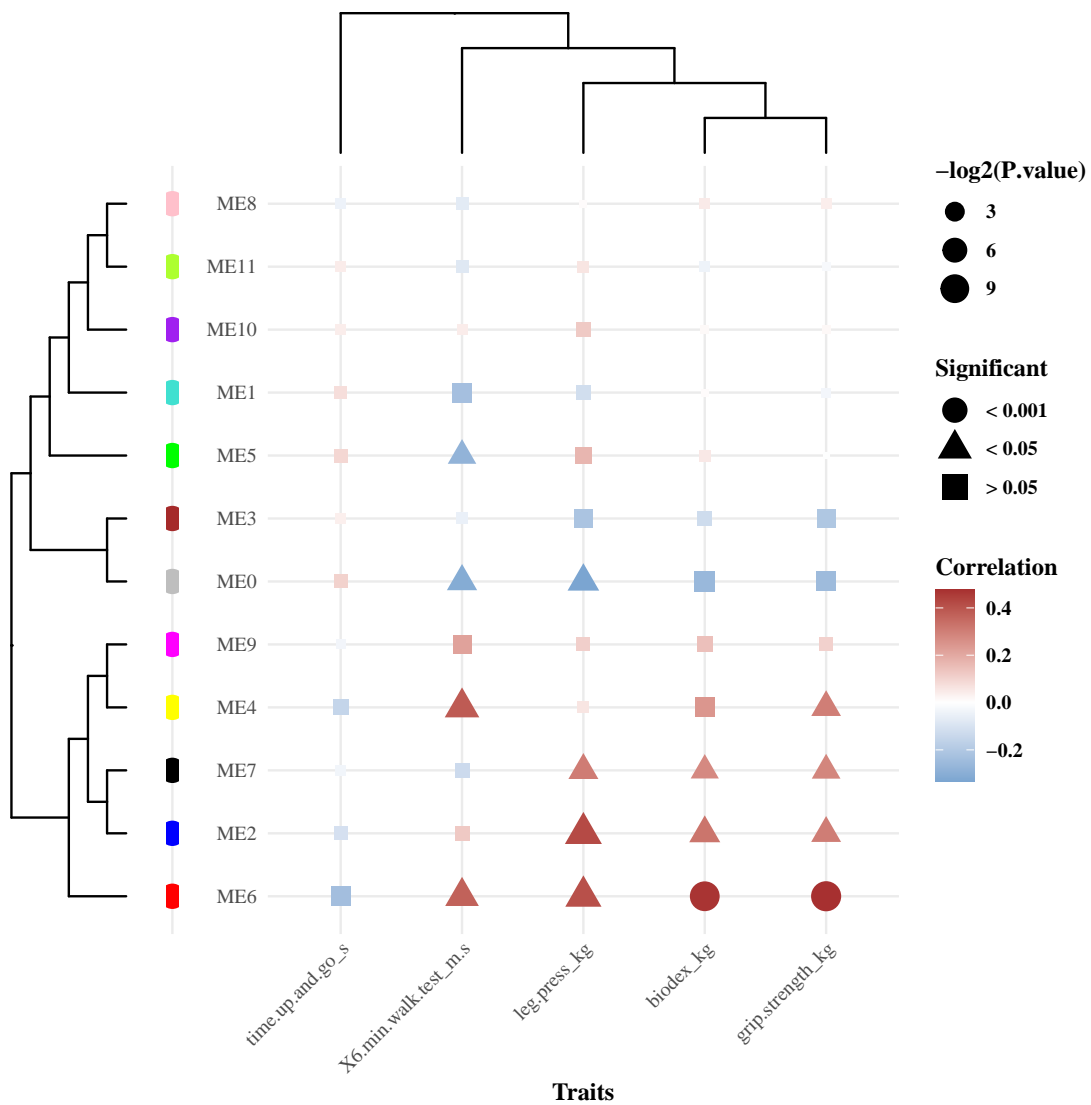
Figure 17: Correlation of gene modules and traits data

Figure 18为图 intersection of genes significant and module memberships 概览。

(**对应文件为 Figure+Table/intersection-of-genes-significant-and-module-memberships.pdf**)
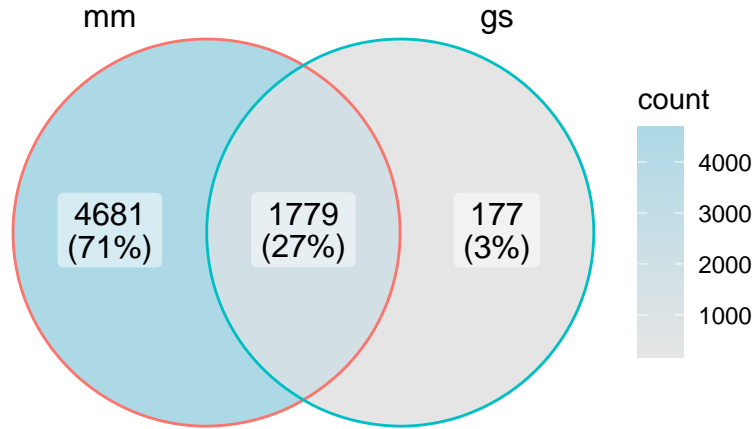
Figure 18: Intersection of genes significant and module memberships

8.6 综合: 结直肠癌和肌少症

8.6.1 （结直肠癌）数据整合

Figure 19为图 all colorectal DEGs 概览。

（对应文件为 `Figure+Table/all-colorectal-DEGs.pdf`）
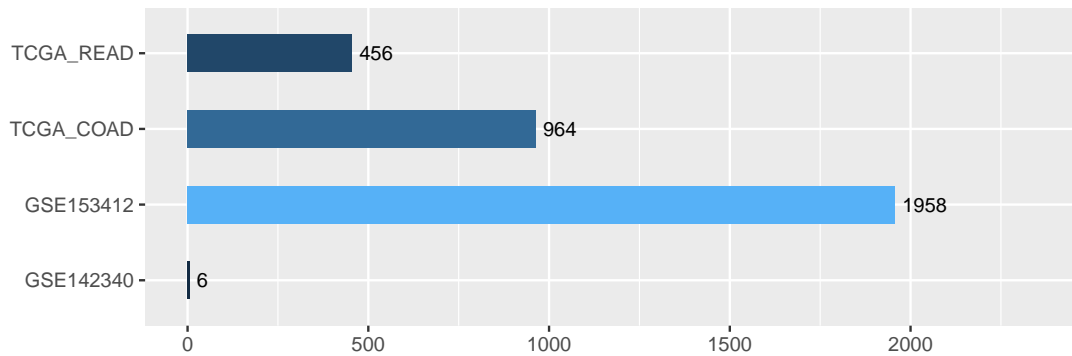
Figure 19: All colorectal DEGs

8.6.2 （结直肠癌与肌少症）交集基因

Figure 20为图 intersection of colorectal DEGs with Sarcopenia significant genes 概览。

（对应文件为 `Figure+Table/intersection-of-colorectal-DEGs-with-Sarcopenia-significant-genes.pdf`）
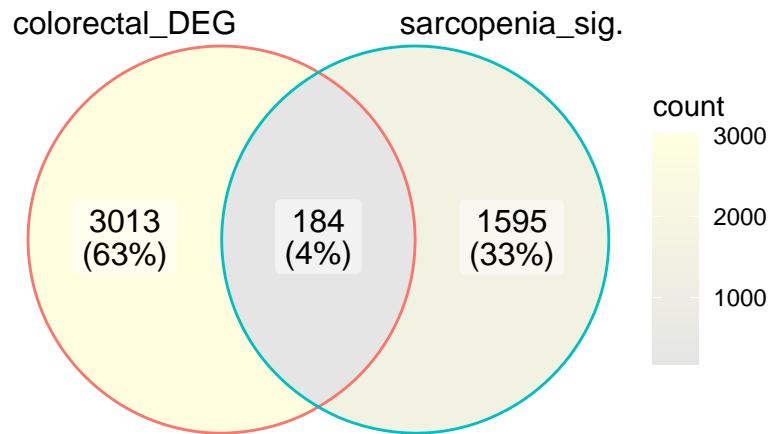
Figure 20: Intersection of colorectal DEGs with Sarcopenia significant genes

8.6.3 富集分析

The Cellular Component (CC), the Molecular Function (MF) and the Biological Process (BP).

Figure 21为图 go enrichment 概览。
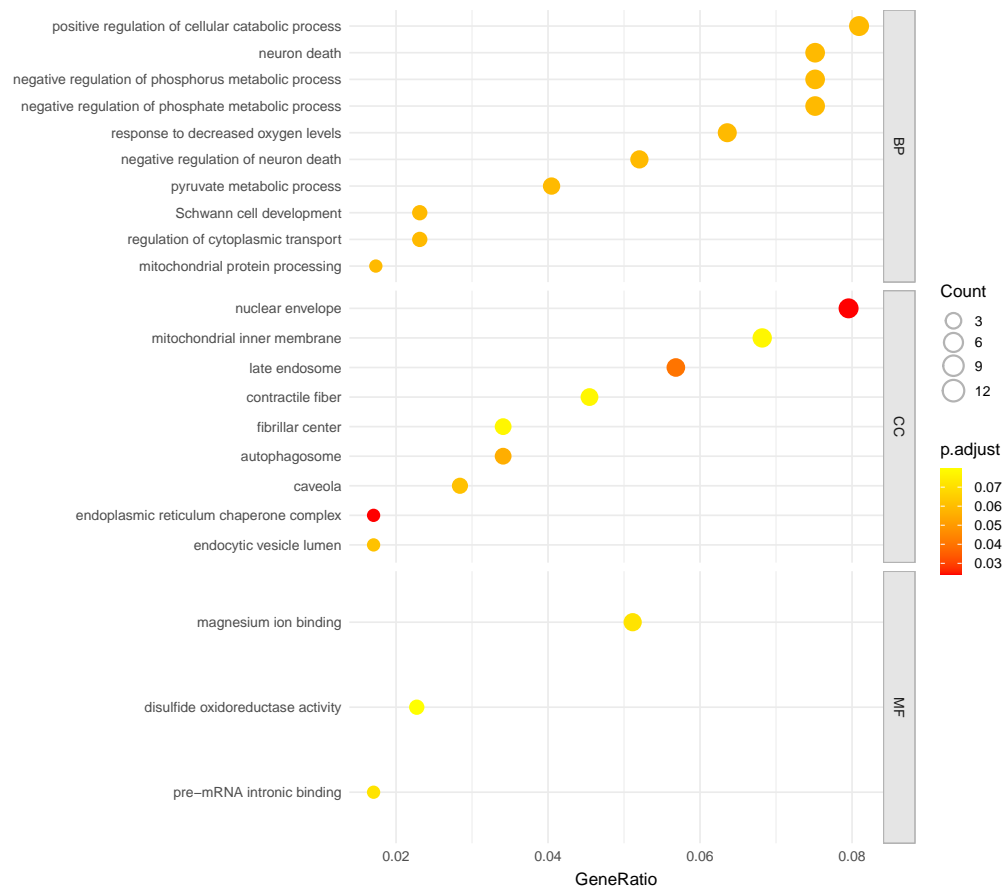
(对应文件为 Figure+Table/go-enrichment.pdf)

Figure 22为图 kegg enrichment 概览。

（对应文件为 Figure+Table/kegg-enrichment.pdf）

Figure 22: Kegg enrichment
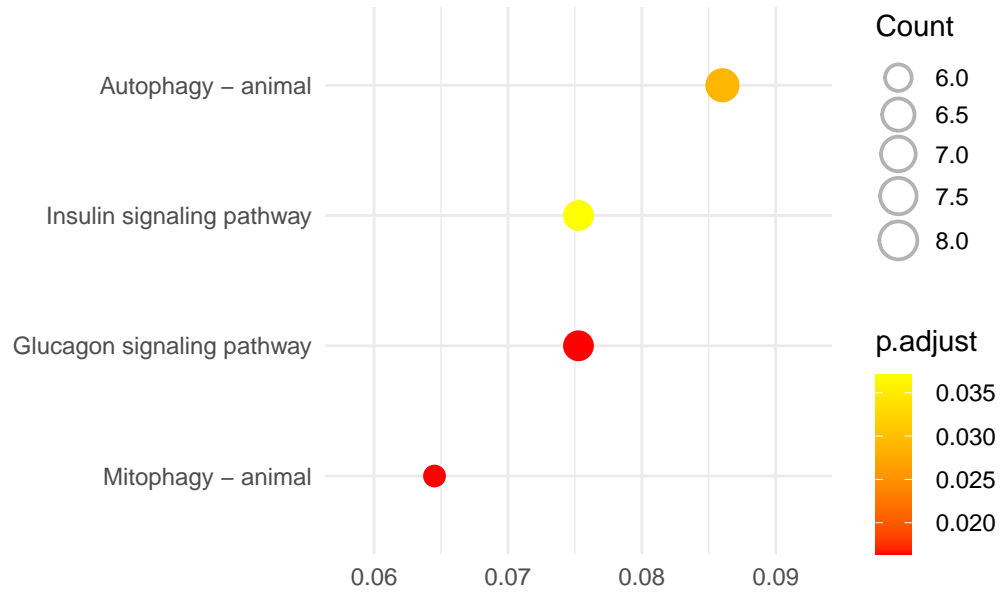
8.6.4 通路可视化

Figure 23为图 hits in autophagy 概览。

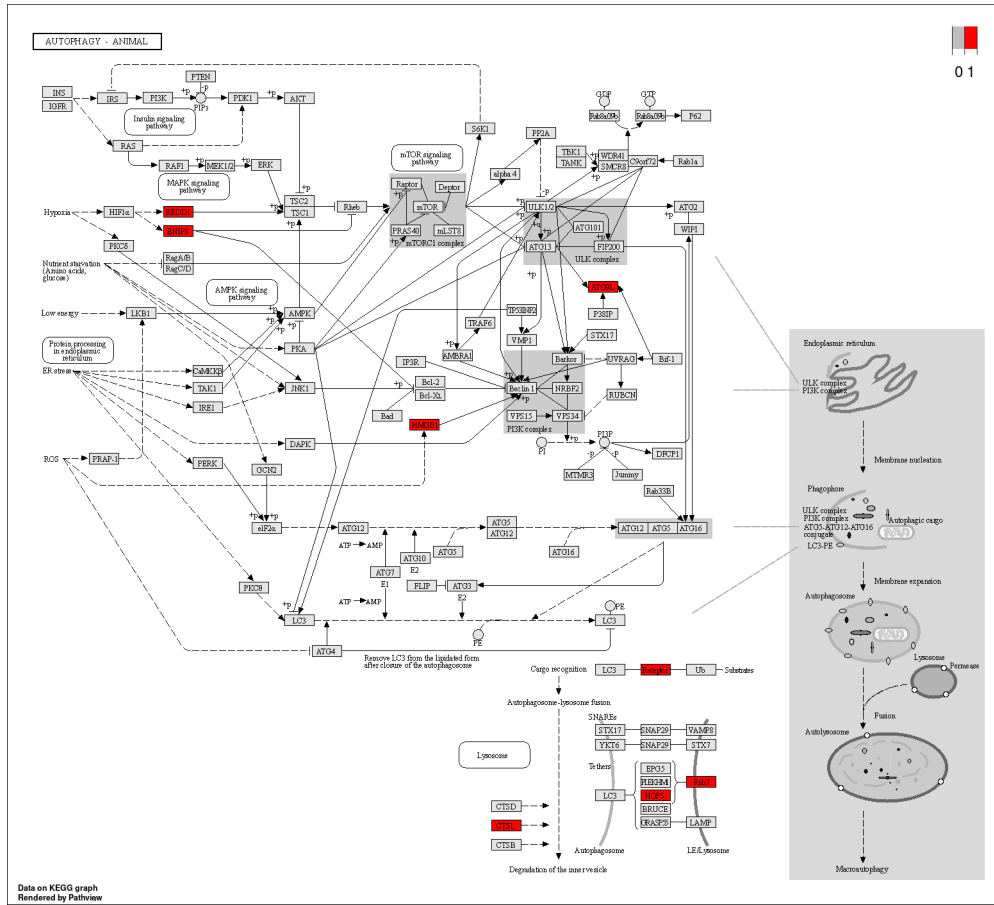**（对应文件为 Figure+Table/hsa04140.pathview.png）**

Figure 23: Hits in autophagy

Figure 24为图 hits in Mitophagy 概览。
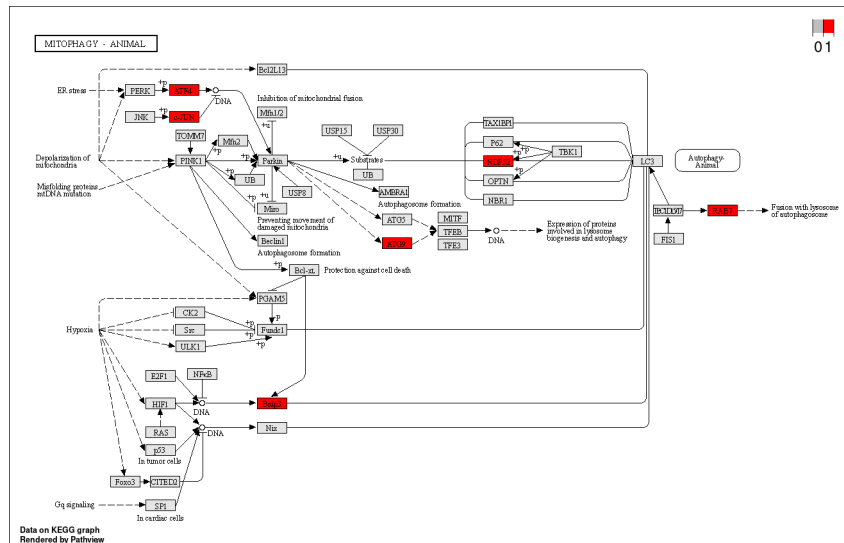
(对应文件为 `Figure+Table/hsa04137.pathview.png`)

Figure 24: Hits in Mitophagy

8.7 验证：线粒体自噬基因是否差异表达

8.7.1 肌少症

Figure 25为图 wilcox test of mitophgy related genes in Sarcopenia 概览。

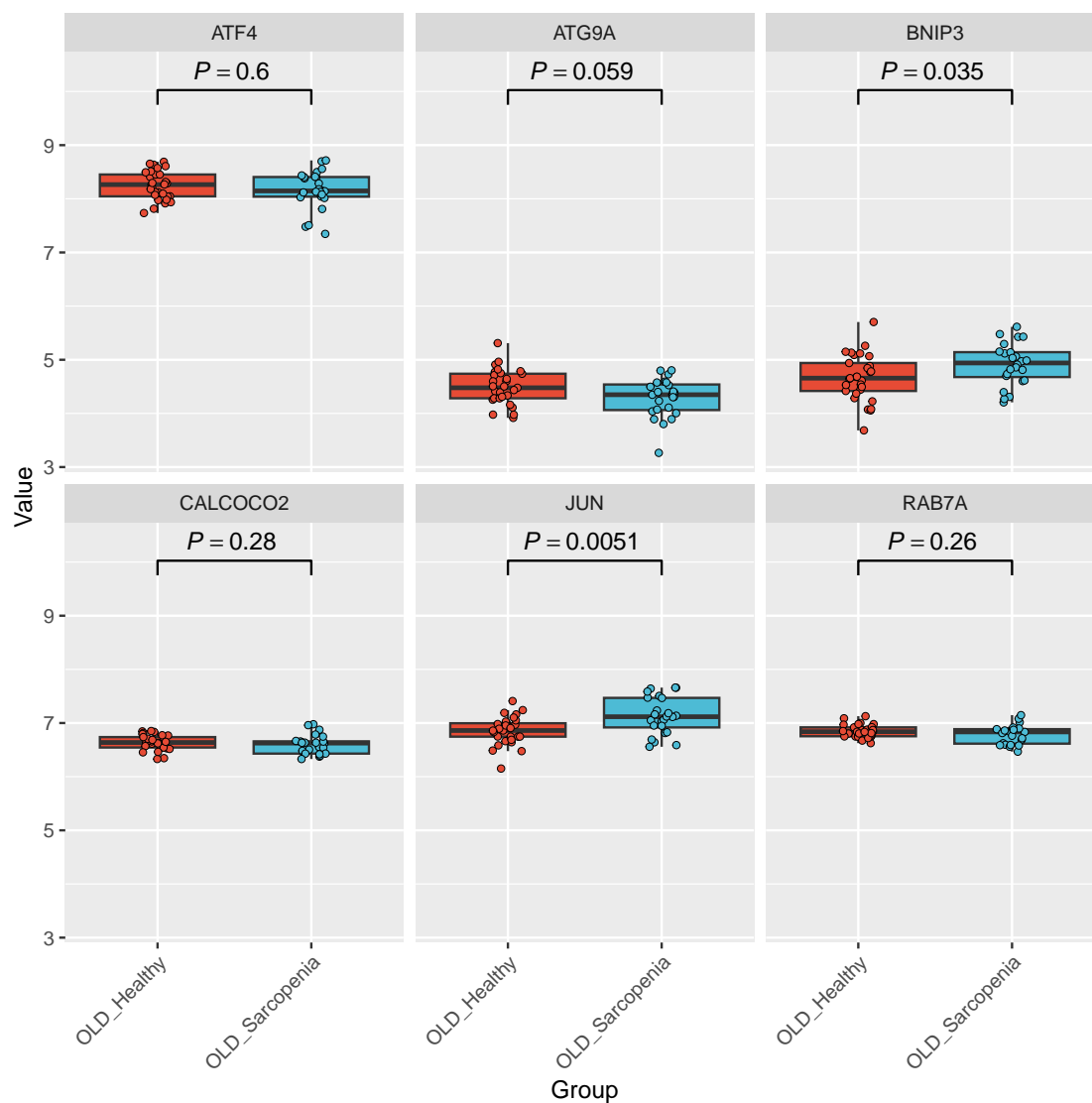**（对应文件为 Figure+Table/wilcox-test-of-mitophgy-related-genes-in-Sarcopenia.pdf）**

Figure 25: Wilcox test of mitophgy related genes in Sarcopenia

8.7.2 结直肠癌

Figure 26为图 wilcox test of mitophgy related genes in READ 概览。

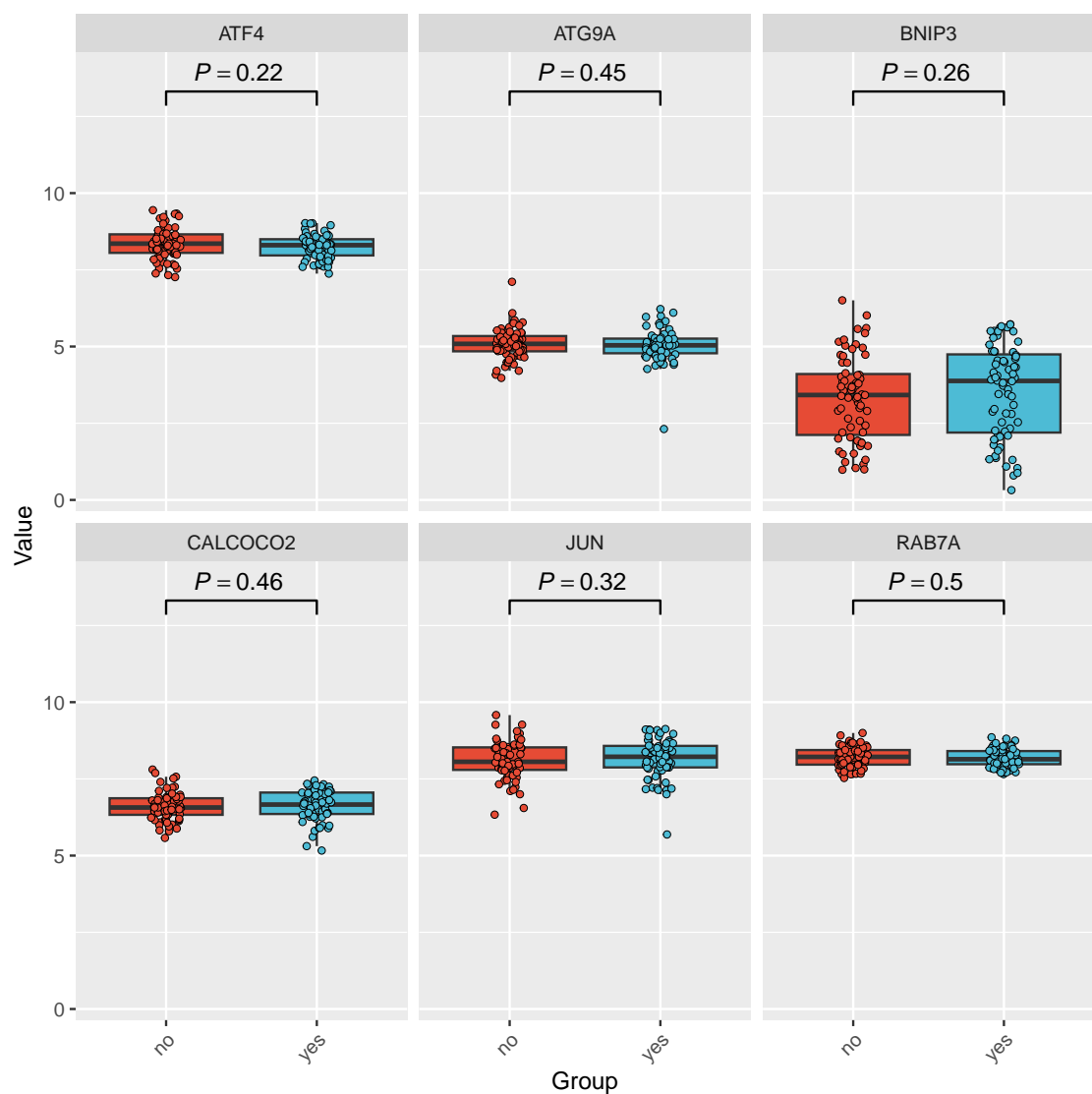**（对应文件为 Figure+Table/wilcox-test-of-mitophgy-related-genes-in-READ.pdf）**

Figure 26: Wilcox test of mitophgy related genes in READ

Figure 27为图 wilcox test of mitophgy related genes in COAD 概览。

(对应文件为 `Figure+Table/wilcox-test-of-mitophgy-related-genes-in-COAD.pdf`)
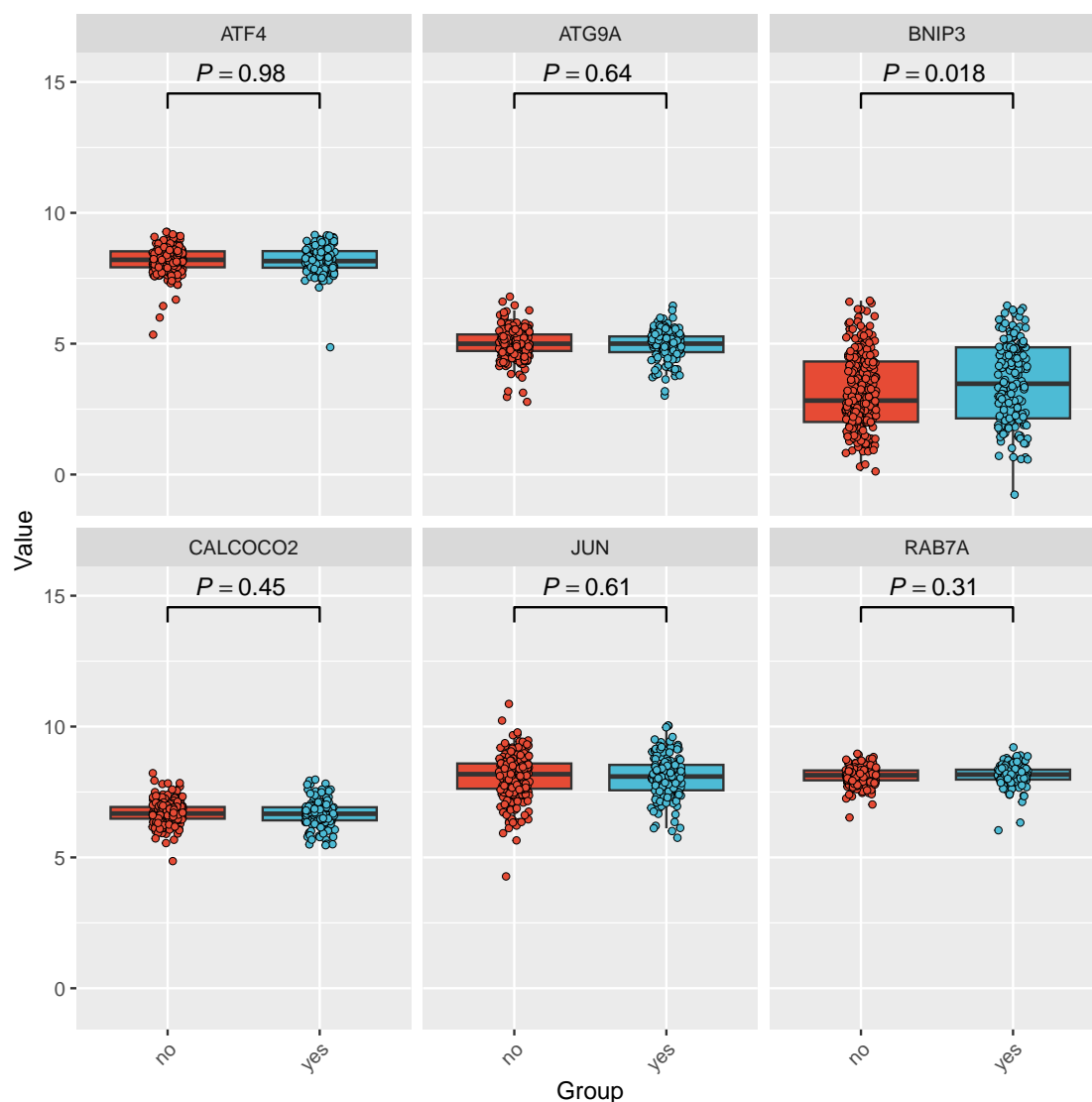
Figure 27: Wilcox test of mitophgy related genes in COAD

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