

# 肺癌和癌旁组织单细胞数据对比分析

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# 1 摘要

- 分析不同组织样本中各细胞比例的情况，包括上皮细胞（肿瘤细胞或正常乳腺细胞）、淋系细胞、髓系细胞等等。
- 对比肺癌与癌旁组织中各细胞的比例，分析在肺癌进展过程中细胞成分的变化，探索在肺癌进展过程中是否出现新的细胞亚群。
- 进化树分析（trajectory analysis）揭示在肺癌进展过程中细胞分化与进化的情况。

# 2 材料和方法

## 2.1 方法

Mainly used method:

- ClusterProfiler used for GSEA enrichment.<sup>1</sup>
- Monocle3 used for cell pseudotime analysis.<sup>2,3</sup>
- Seurat used for scRNA-seq processing; SCSA used for cell type annotation.<sup>4-6</sup>
- Seurat used for spatial scRNA-seq analysis.<sup>4,5</sup>
- Other R packages used for statistic analysis or data visualization.

# 3 分析结果

- 不同组织样本细胞比例如图所示 (Fig. 1)。（a、c、d 为癌组织切片； b、e 为癌旁组织切片）。癌旁组织同样发生了大量的巨噬细胞浸润，整体比例甚至高于癌组织切片。对癌组织的癌细胞分化来源分析，占据癌细胞比例的为上皮细胞和基底细胞 (Epithelial cells、basal cells)。
- 根据 Fig. 1 两组织切片的共有细胞，随后，本研究主要分析了免疫细胞的巨噬细胞的亚类 (Fig. 2)。根据拟时分析和细胞重聚类 (Fig. 2b、c)，将巨噬细胞分为三种类型：M1、M2、M3。M3、M2 为癌组织切片中的巨噬细胞，M1 为癌旁组织切片的巨噬细胞，它们的差异基因 (Fig. 2d) 的富集通路如 Fig. 2 e (M3 vs M1) 、f (M2 vs M1) 、g (M3 vs M2)。其中，“Lysosome”、“Spliceosome”、“Phagosome”对应通路可能是三者功能的重要区分。
- 对癌细胞的进化树分析如图 (Epithelial cell -> Cancer cell) (Fig. 3)。癌旁上皮细胞向癌细胞的发展分化 (拟时轨迹)，通路“ATP-dependent chromatin remodeling”、“Rap1 signaling pathway”、“Spliceosome”发生显著变化。(Fig. 3 d 为拟时热图)

Figure 1 (下方图) 为图 MAIN fig1 cells proportion 概览。

(对应文件为 ./Figure+Table/fig1.pdf)

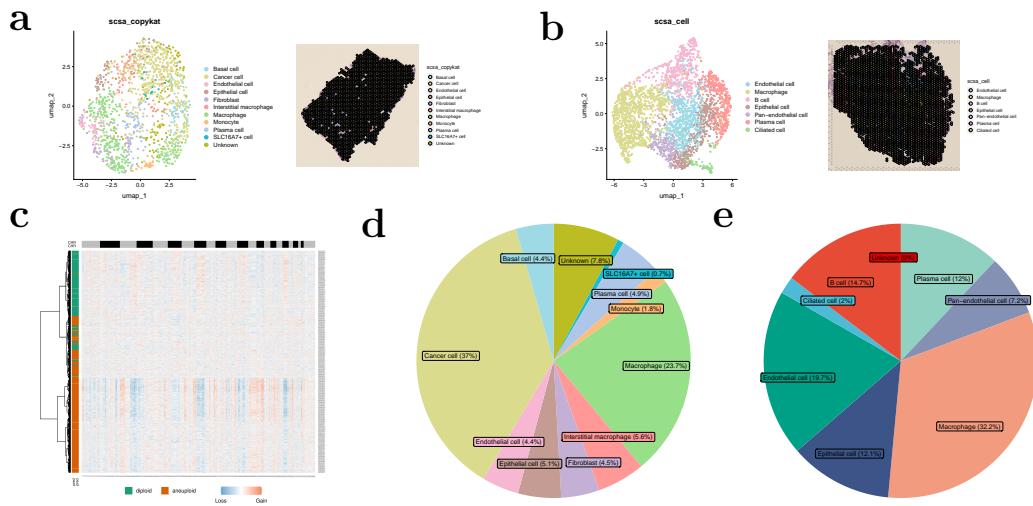


Figure 1: MAIN fig1 cells proportion

Figure 2 (下方图) 为图 MAIN fig2 Macrophage subtypes analysis 概览。

(对应文件为 ./Figure+Table/fig2.pdf)

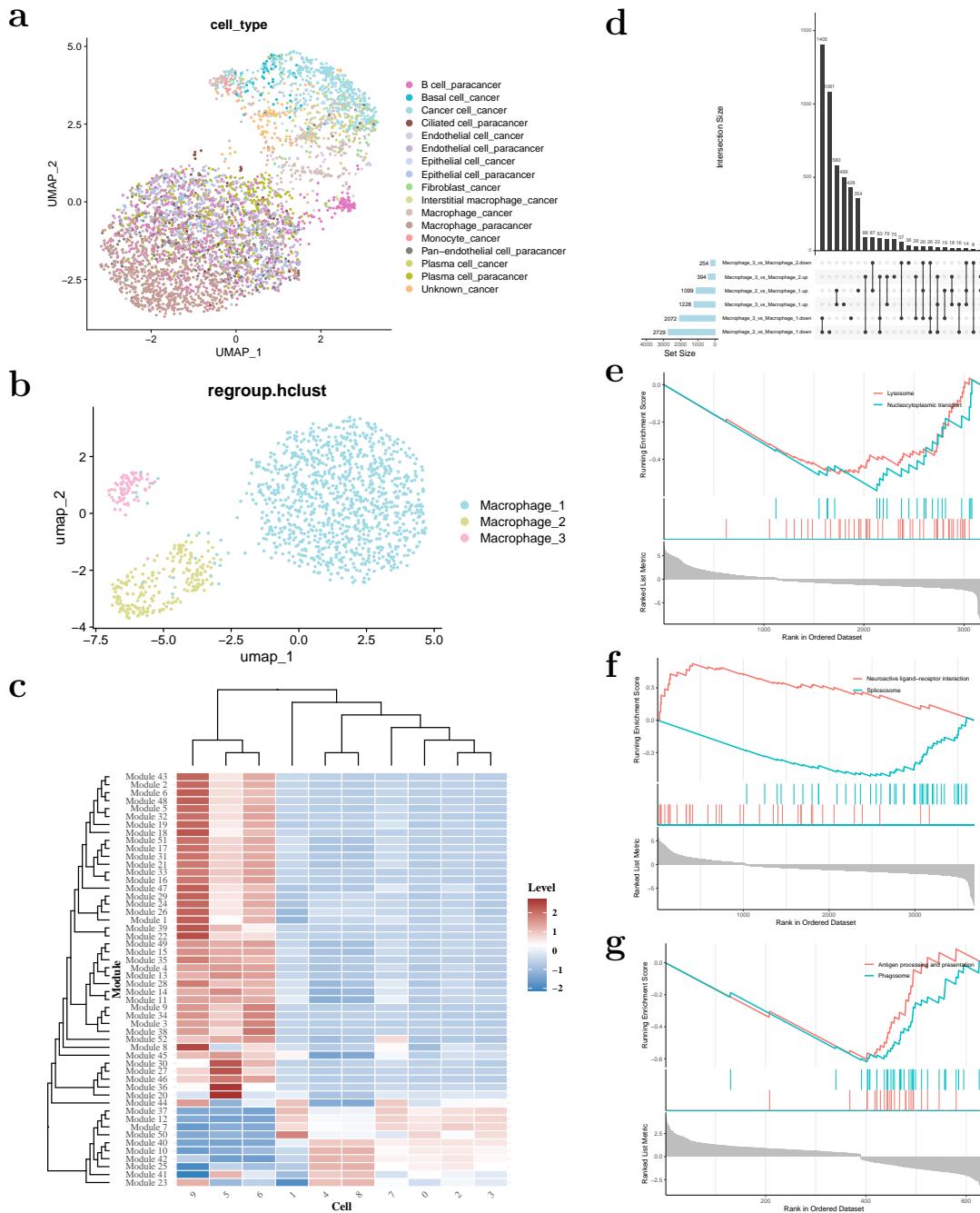


Figure 2: MAIN fig2 Macrophage subtypes analysis

Figure 3 (下方图) 为图 MAIN fig3 Trajectory analysis of cancer cells 概览。

(对应文件为 ./Figure+Table/fig3.pdf)

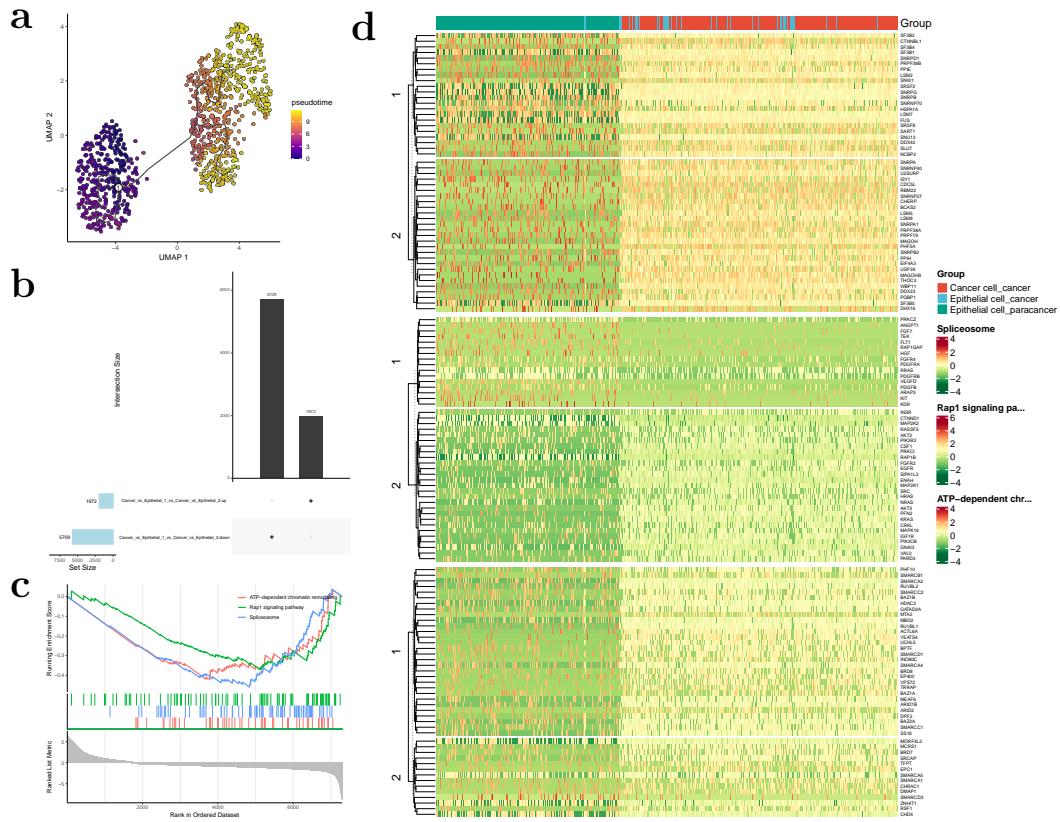


Figure 3: MAIN fig3 Trajectory analysis of cancer cells

## 4 附：分析流程

### 4.1 癌组织切片分析

#### 4.1.1 空间转录组数据前处理与可视化

使用 Seurat 前处理空间转录组数据集，完成降维聚类，使用 SCSA 对细胞进行注释。

Figure 4 (下方图) 为图 Cancer tissue SCSA annotation 概览。

(对应文件为 Figure+Table/Cancer-tissue-SCSA-annotation.pdf)

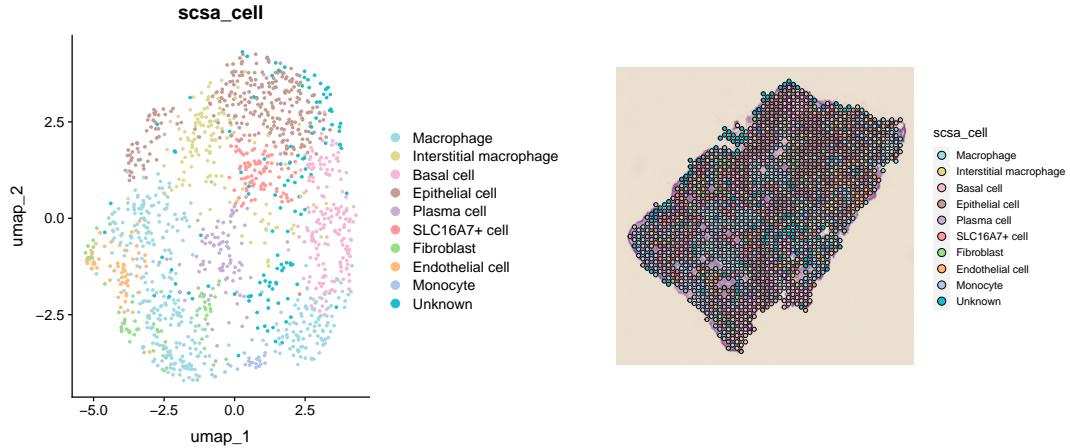


Figure 4: Cancer tissue SCSA annotation

#### 4.1.2 癌细胞鉴定

- 使用 copyKAT 鉴定癌细胞。

Figure 5 (下方图) 为图 Cancer tissue copyKAT prediction of aneuploidy 概览。

(对应文件为 Figure+Table/copykat\_heatmap.png)

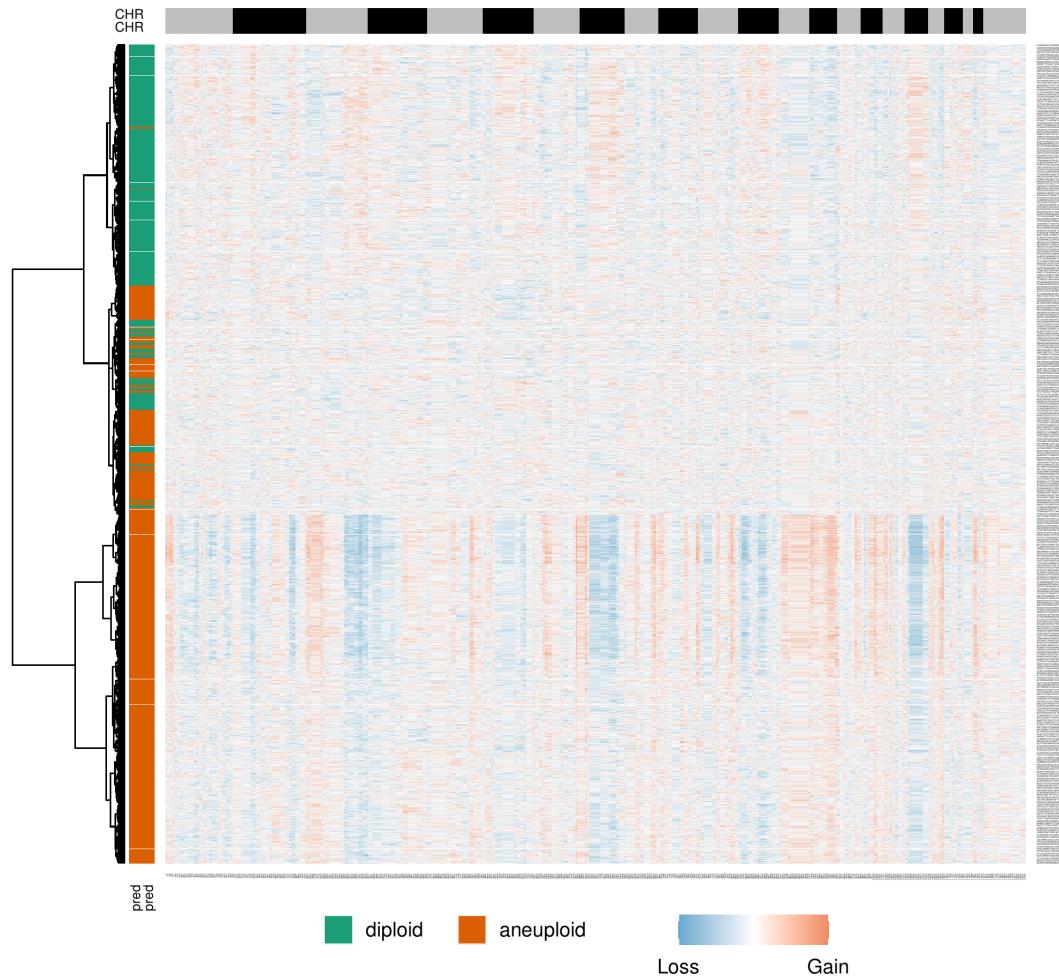


Figure 5: Cancer tissue copyKAT prediction of aneuploidy

Figure 6 (下方图) 为图 Cancer tissue cell mapped of copyKAT prediction 概览。

(对应文件为 [Figure+Table/Cancer-tissue-cell-mapped-of-copyKAT-prediction.pdf](#))

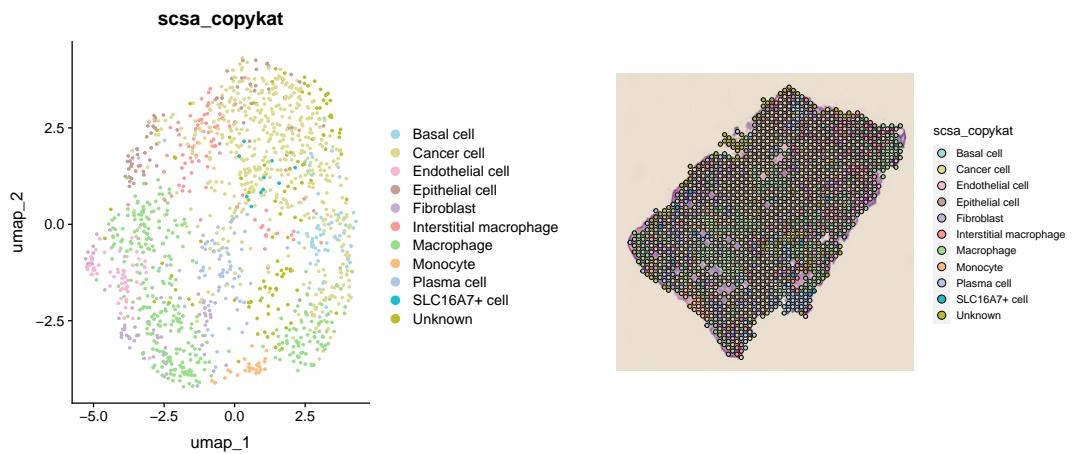


Figure 6: Cancer tissue cell mapped of copyKAT prediction

#### 4.1.3 细胞比例分析

Figure 7 (下方图) 为图 Cancer tissue cell proportion 概览。

(对应文件为 Figure+Table/Cancer-tissue-cell-proportion.pdf)

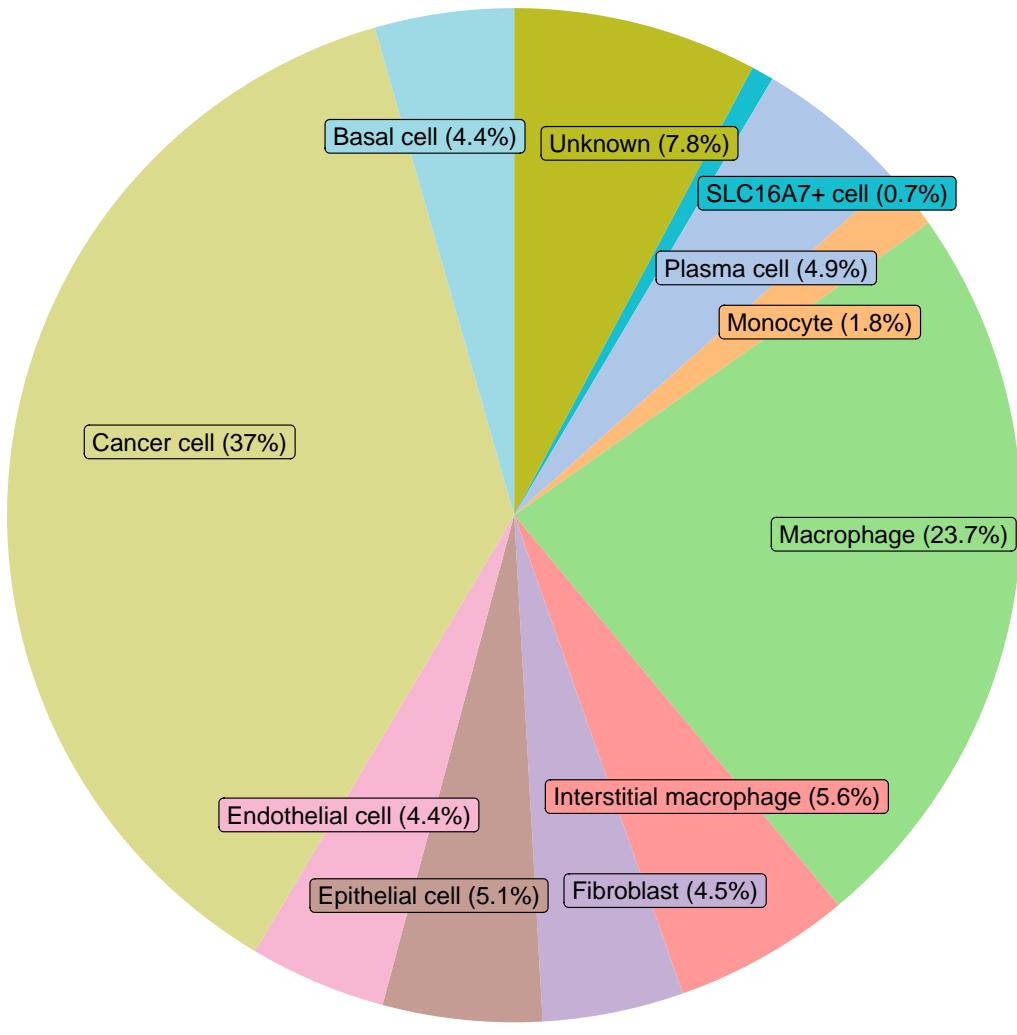


Figure 7: Cancer tissue cell proportion

## 4.2 癌旁组织切片分析

### 4.2.1 空间转录组数据前处理与可视化

使用 Seurat 前处理空间转录组数据集，完成降维聚类，使用 SCSA 对细胞进行注释。

Figure 8 (下方图) 为图 Paracancerous tissue SCSA annotation 概览。

(对应文件为 Figure+Table/Paracancerous-tissue-SCSA-annotation.pdf)

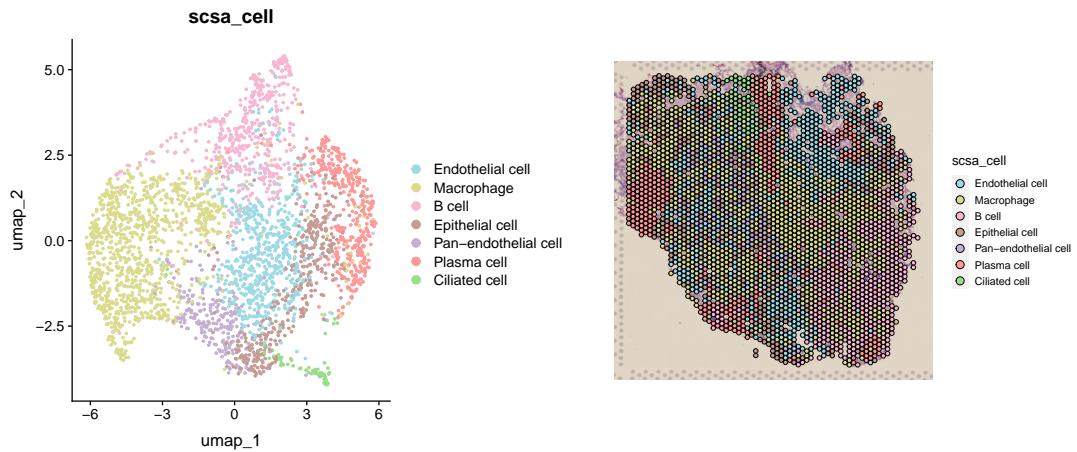


Figure 8: Paracancerous tissue SCSA annotation

#### 4.2.2 细胞比例分析

Figure 9 (下方图) 为图 Paracancerous tissue cell proportion 概览。

(对应文件为 Figure+Table/Paracancerous-tissue-cell-proportion.pdf)

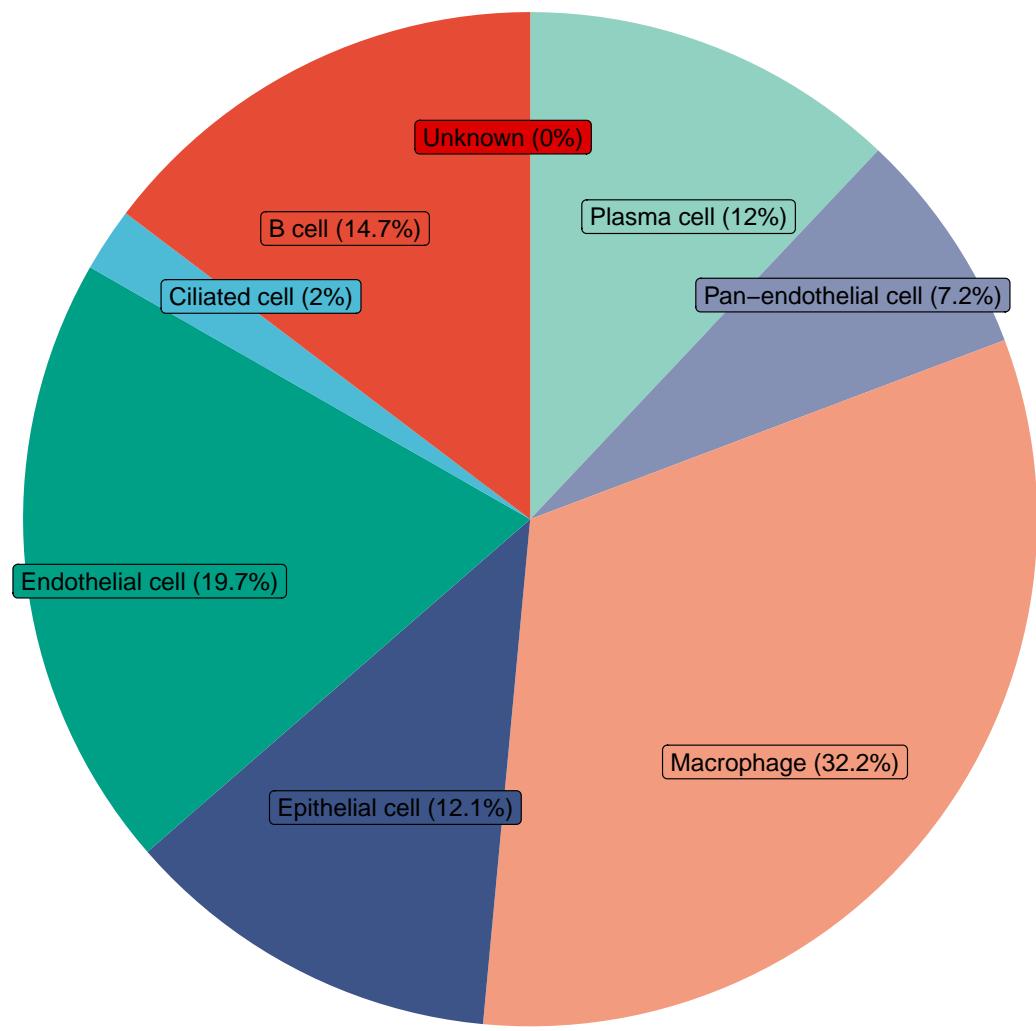


Figure 9: Paracancerous tissue cell proportion

### 4.3 癌组织和癌旁组织整合分析

#### 4.3.1 集成癌组织和癌旁组织数据

Figure 10 (下方图) 为图 Integrated The cell type 概览。

(对应文件为 Figure+Table/Integrated-The-cell-type.pdf)

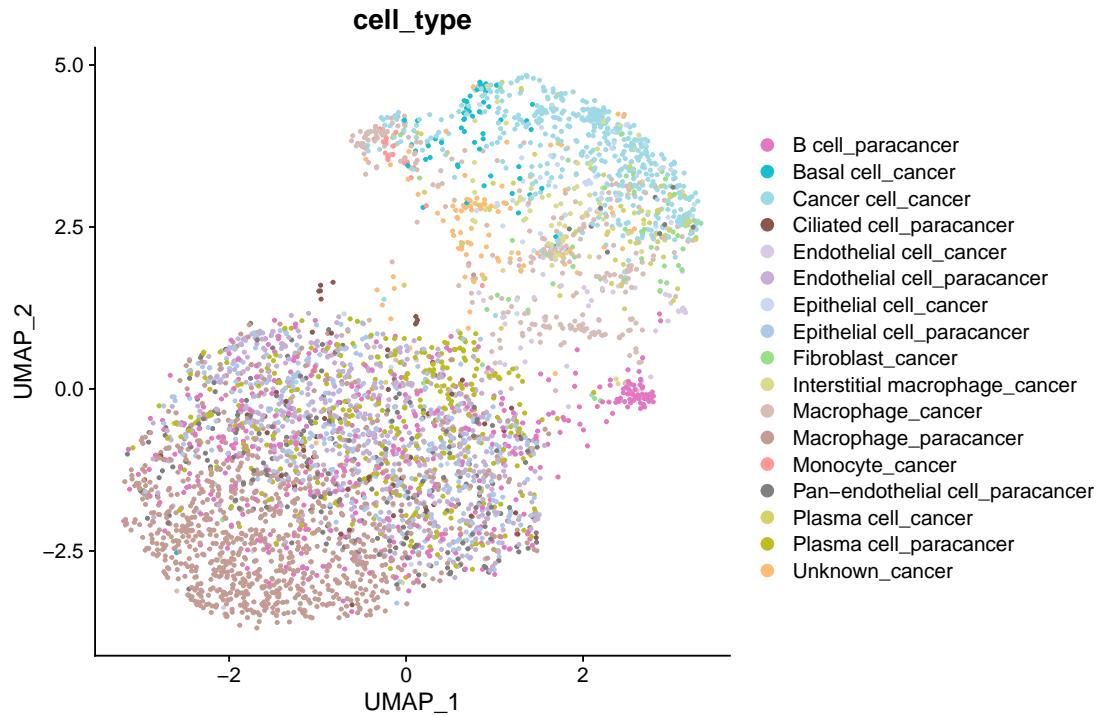


Figure 10: Integrated The cell type

#### 4.3.2 巨噬细胞的亚群分析

Figure 11 (下方图) 为图 Macrophage subtypes The regroup hclust 概览。

(对应文件为 Figure+Table/Macrophage-subtypes-The-regroup-hclust.pdf)

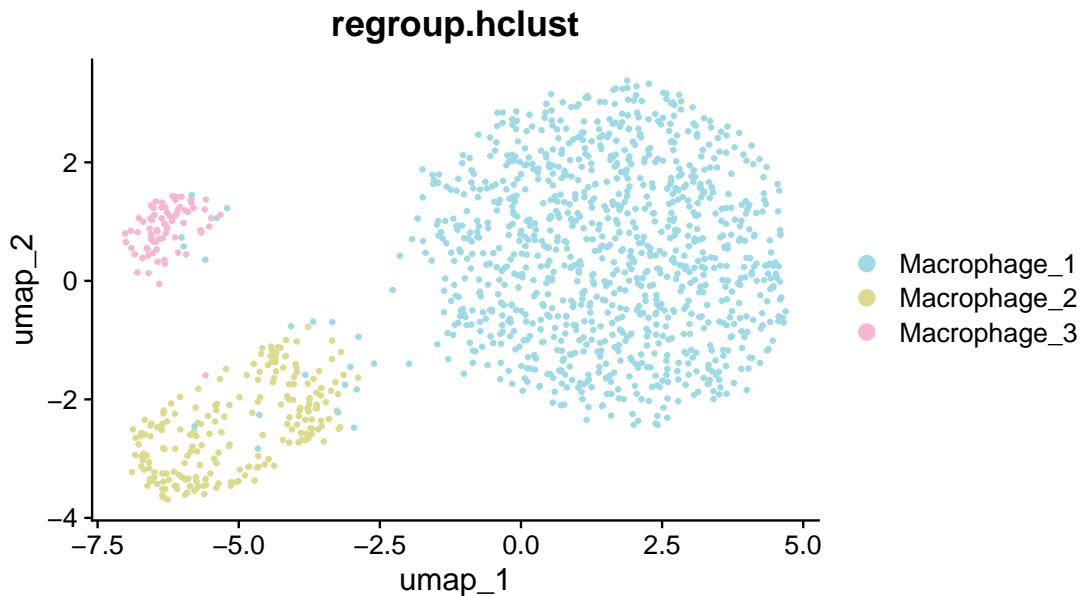


Figure 11: Macrophage subtypes The regroup hclust

Figure 12 (下方图) 为图 Macrophage subtypes gene module heatmap 概览。

(对应文件为 Figure+Table/Macrophage-subtypes-gene-module-heatmap.pdf)

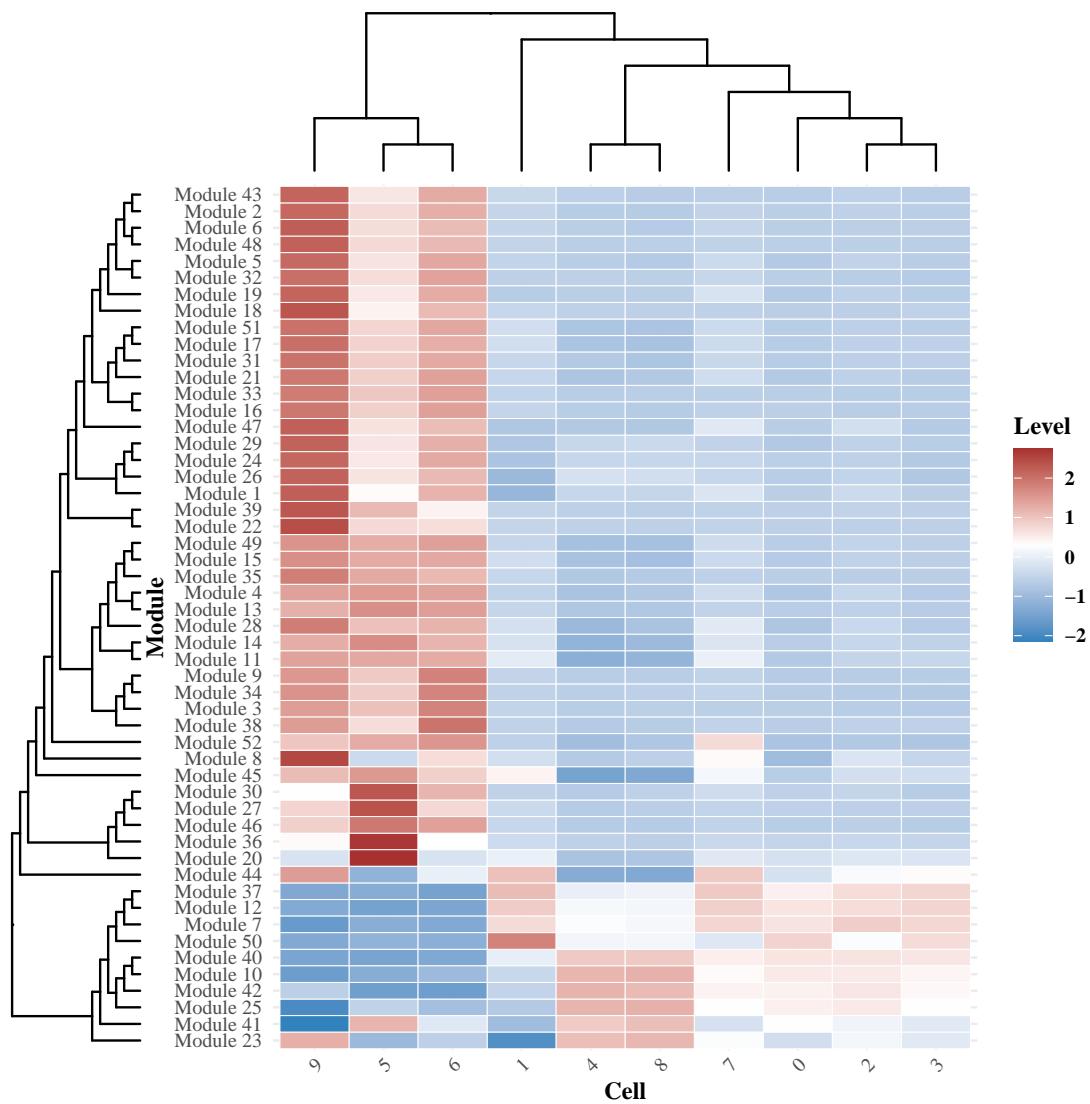


Figure 12: Macrophage subtypes gene module heatmap

#### 4.3.3 巨噬细胞亚群间差异分析

Figure 13 (下方图) 为图 Macrophage subtypes contrasts DEGs intersection 概览。

(对应文件为 Figure+Table/Macrophage-subtypes-contrasts-DEGs-intersection.pdf)

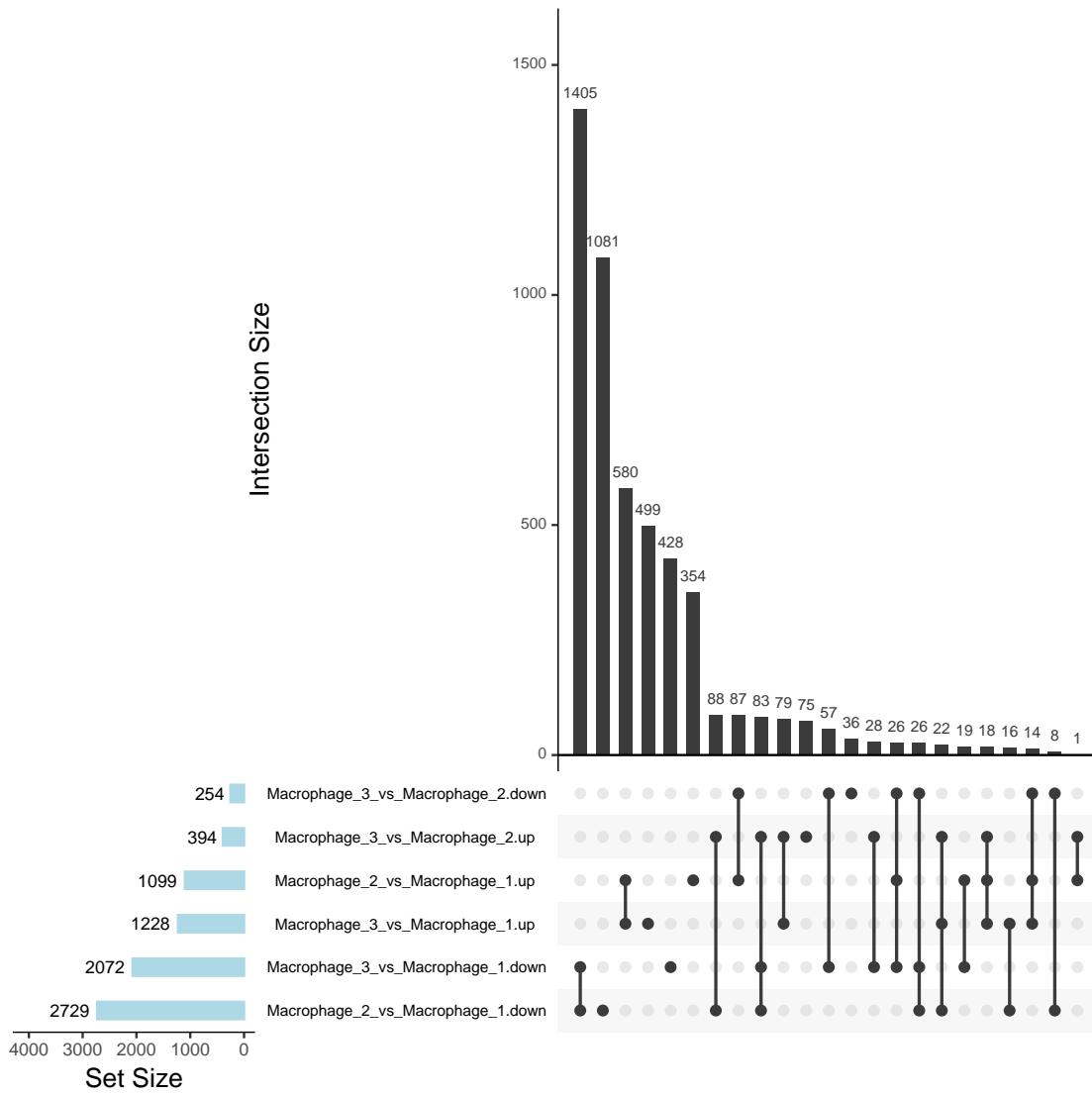


Figure 13: Macrophage subtypes contrasts DEGs intersection

#### 4.3.4 巨噬细胞亚群间差异功能分析

**4.3.4.1 M3 vs M1** Figure 14 (下方图) 为图 Macrophage 3 vs Macrophage 1 GSEA plot of the pathways 概览。

(对应文件为 Figure+Table/Macrophage-3-vs-Macrophage-1-GSEA-plot-of-the-pathways.pdf)

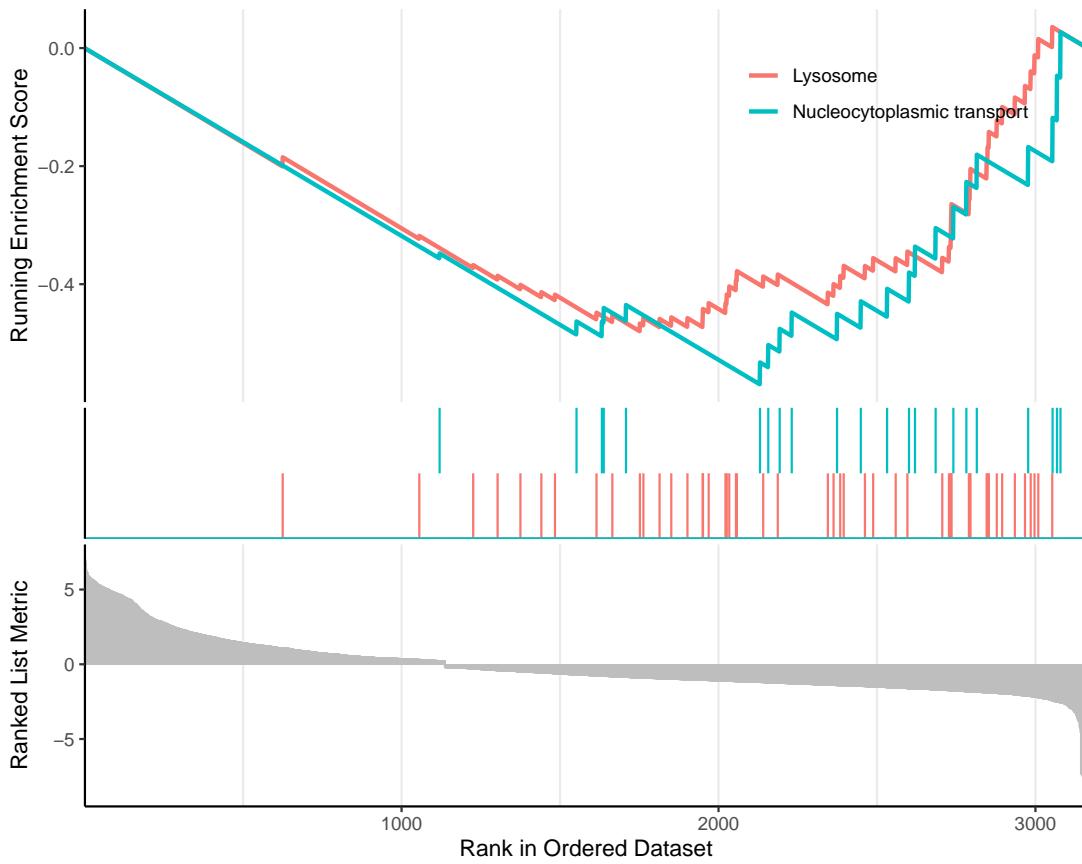


Figure 14: Macrophage 3 vs Macrophage 1 GSEA plot of the pathways

**4.3.4.2 M2 vs M1** Figure 15 (下方图) 为图 Macrophage 2 vs Macrophage 1 GSEA plot of the pathways 概览。

(对应文件为 Figure+Table/Macrophage-2-vs-Macrophage-1-GSEA-plot-of-the-pathways.pdf)

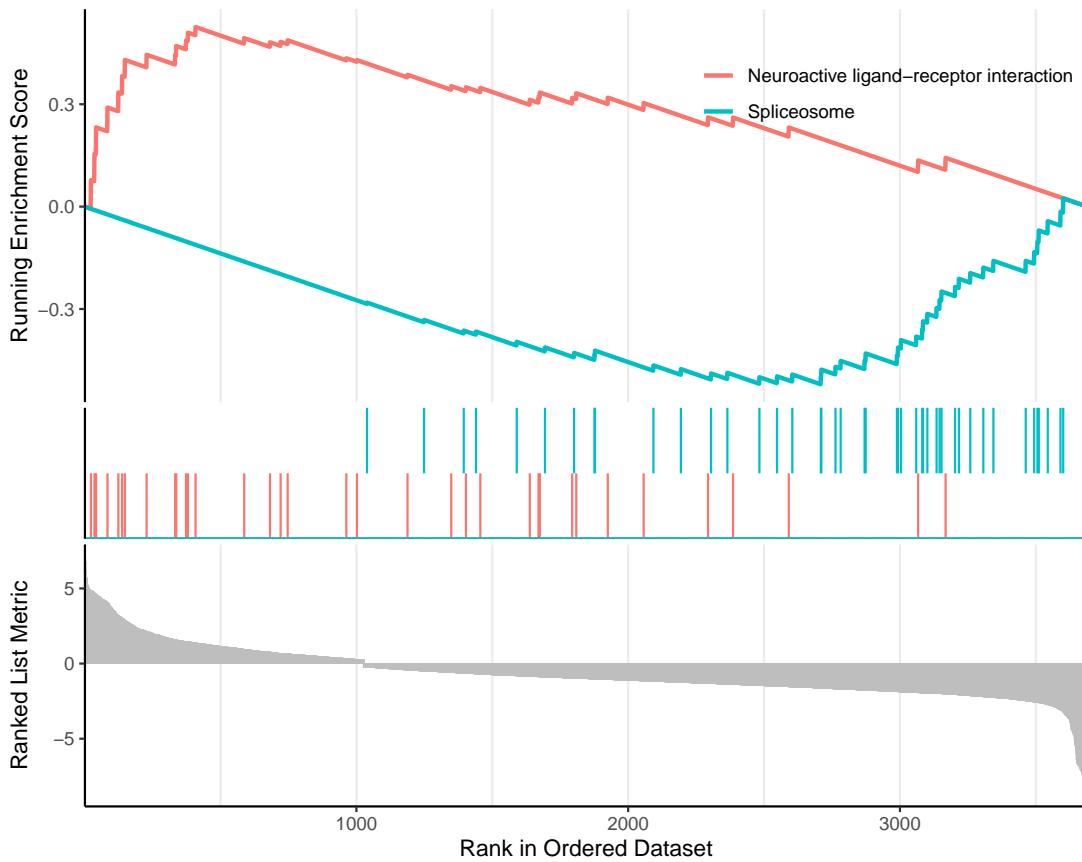


Figure 15: Macrophage 2 vs Macrophage 1 GSEA plot of the pathways

**4.3.4.3 M3 vs M2** Figure 16 (下方图) 为图 Macrophage 3 vs Macrophage 2 GSEA plot of the pathways 概览。

(对应文件为 Figure+Table/Macrophage-3-vs-Macrophage-2-GSEA-plot-of-the-pathways.pdf)

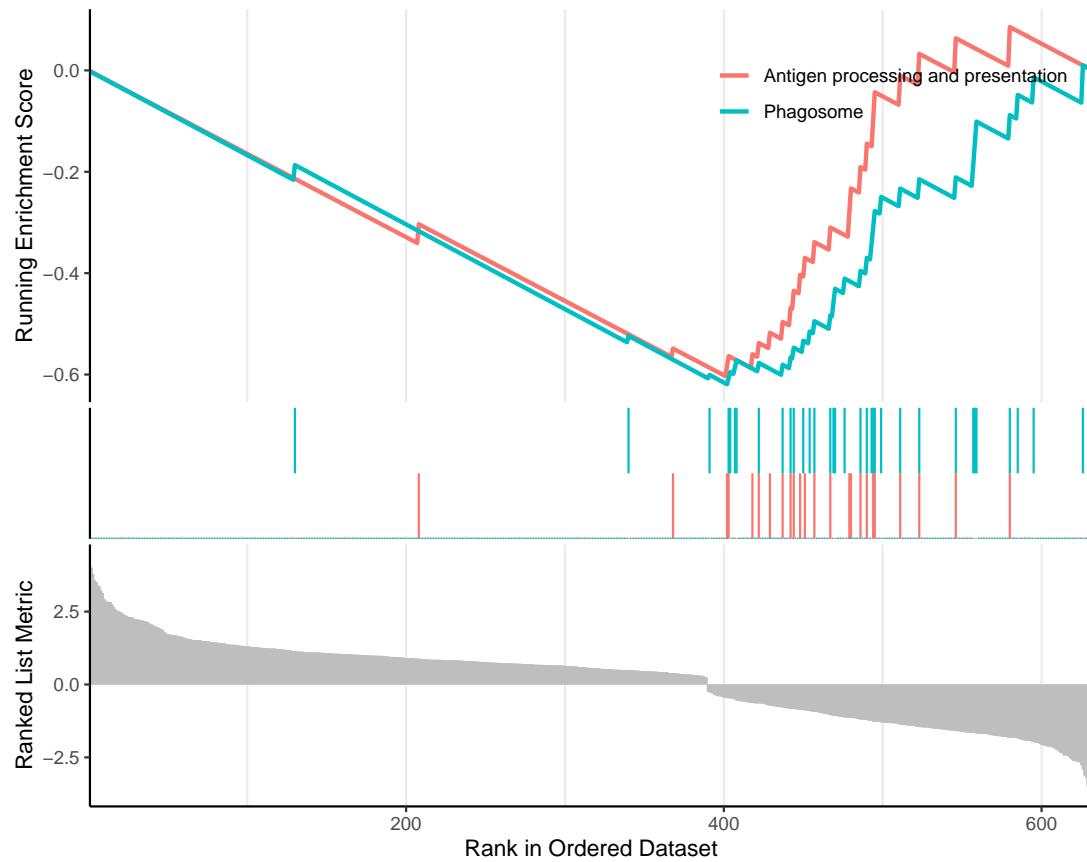


Figure 16: Macrophage 3 vs Macrophage 2 GSEA plot of the pathways

#### 4.3.5 癌细胞进化树分析 (trajectory analysis)

4.3.5.1 上皮细胞向癌细胞的衍化 Figure 17 (下方图) 为图 cancer trajectory pseudotime 概览。

(对应文件为 Figure+Table/cancer-trajectory-pseudotime.pdf)

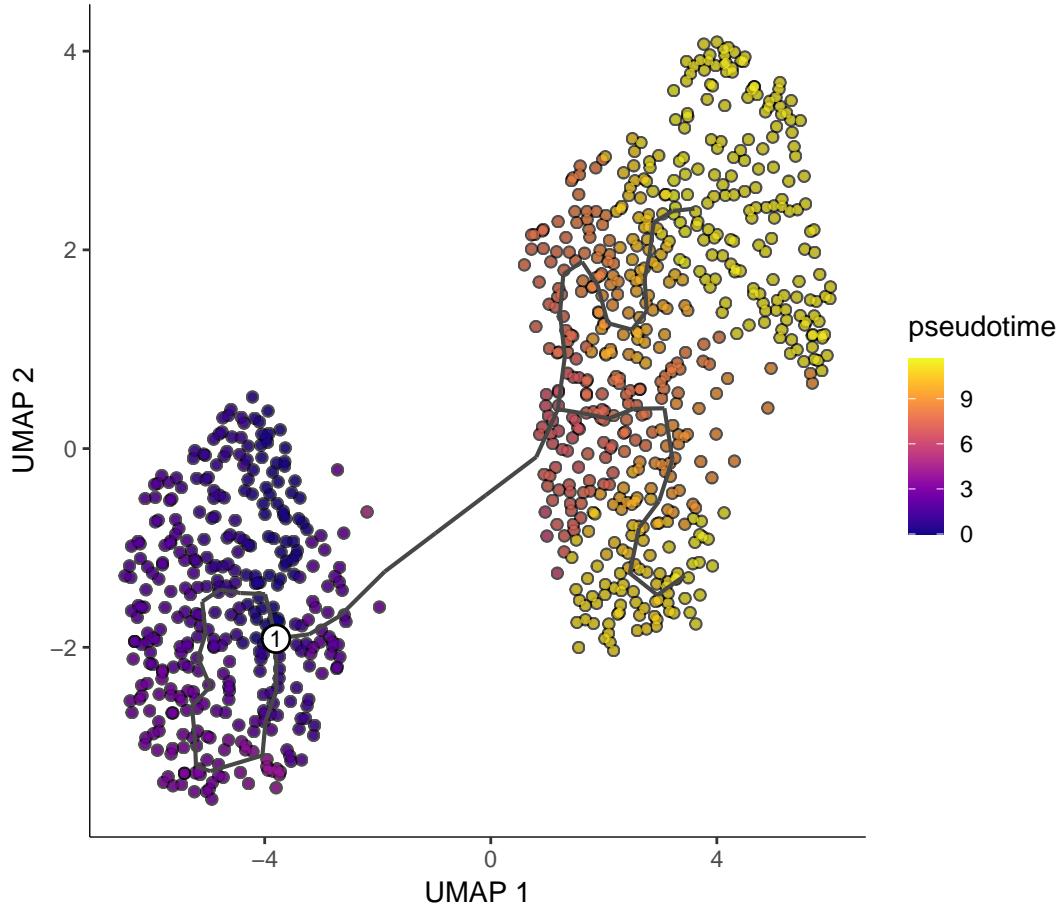


Figure 17: Cancer trajectory pseudotime

**4.3.5.2 上皮细胞和癌细胞的差异分析** Figure 18 (下方图) 为图 Cancer trajectory contrasts DEGs intersection 概览。

(对应文件为 Figure+Table/Cancer-trajectory-contrasts-DEGs-intersection.pdf)

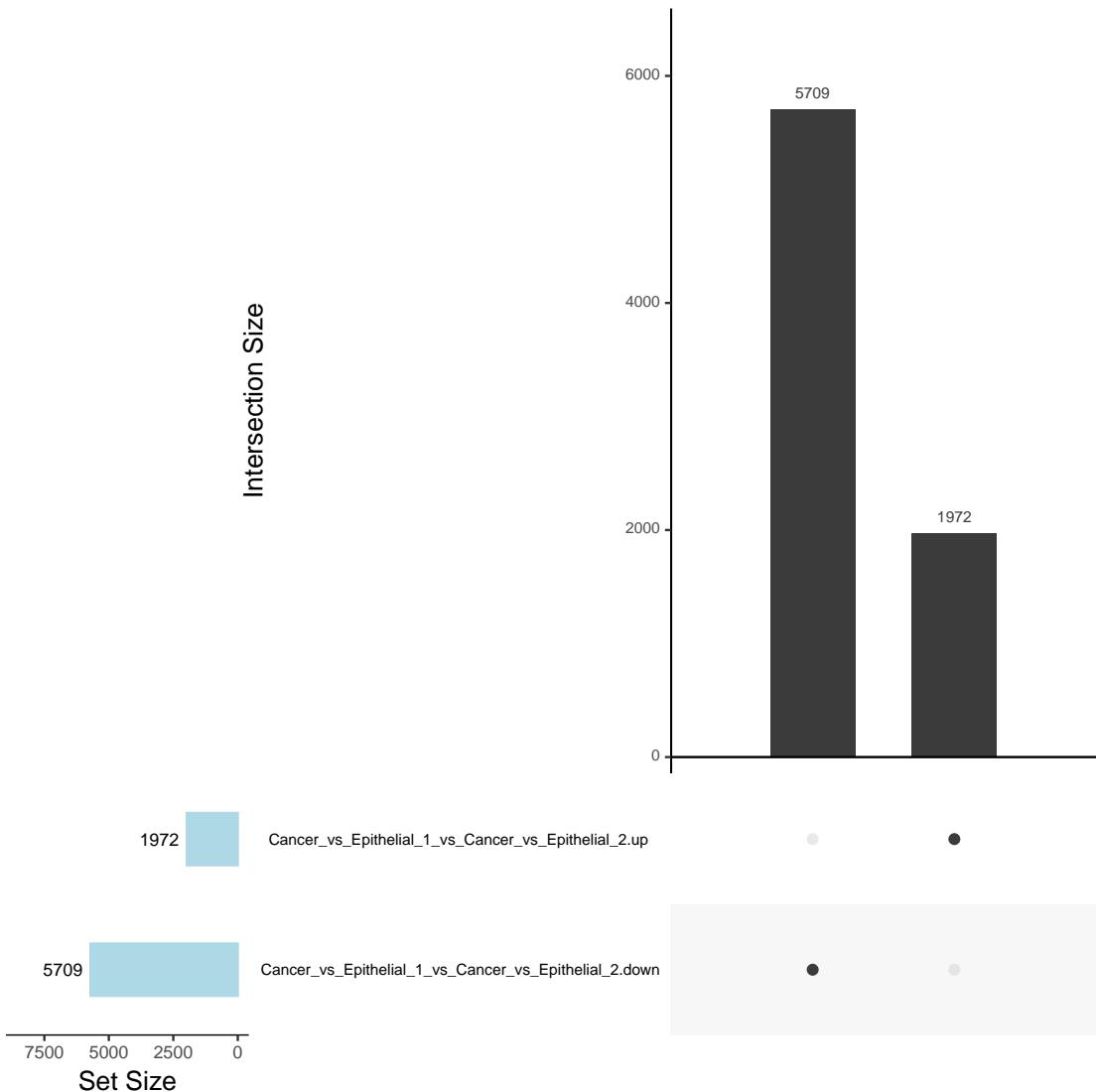


Figure 18: Cancer trajectory contrasts DEGs intersection

**4.3.5.3 上皮细胞和癌细胞的差异的功能分析** Figure 19 (下方图) 为图 Cancer vs Epithelial GSEA plot of the pathways 概览。

(对应文件为 Figure+Table/Cancer-vs-Epithelial-GSEA-plot-of-the-pathways.pdf)

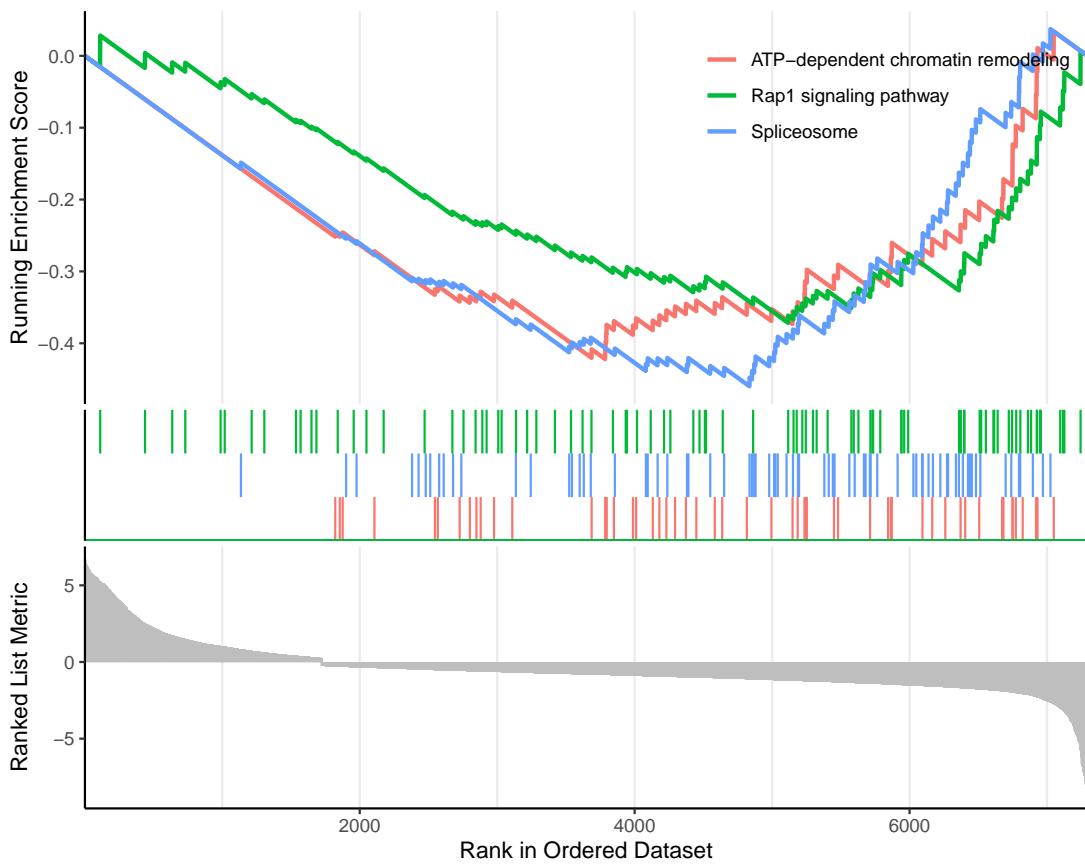


Figure 19: Cancer vs Epithelial GSEA plot of the pathways

Figure 20 (下方图) 为图 Cancer trajectory show pathway genes in pseudotime 概览。

(对应文件为 [Figure+Table/Cancer-trajectory-show-pathway-genes-in-pseudotime.pdf](#))

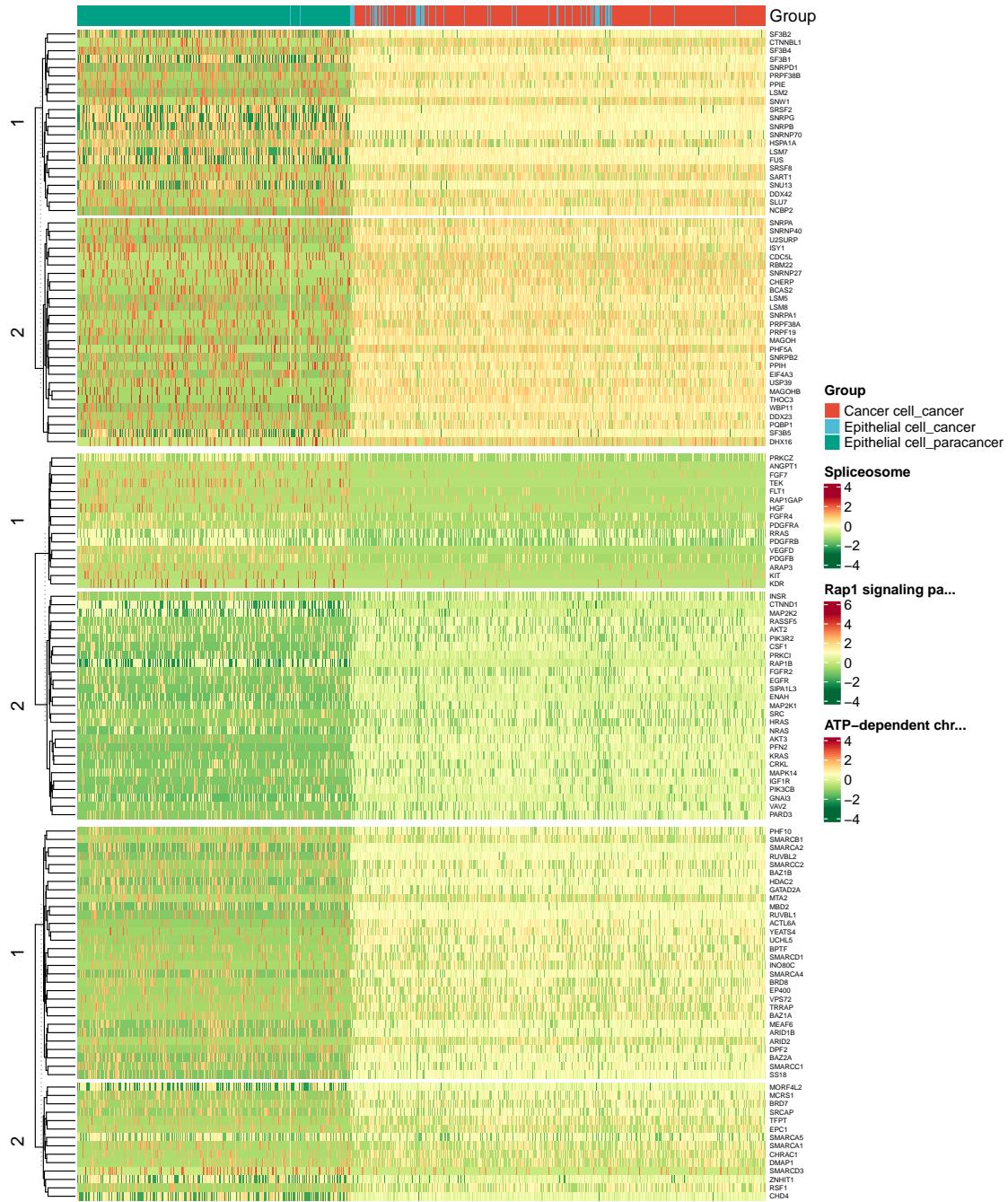


Figure 20: Cancer trajectory show pathway genes in pseudotime

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- Qiu, X. *et al.* Reversed graph embedding resolves complex single-cell trajectories. *Nature Methods* **14**, (2017).

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