Teesside University
School of Health & Life Sciences
tees.ac.uk/health&lifesciences

Identifying Potential Therapeutic Targets within Different Glioblastoma Cell Types using Single-Cell Sequencing

Rayan Ahmed C2168667@tees.ac.uk, Dr Xinzhong Li x.li@tees.ac.uk
MSc Bioinformatics

1. Introduction

- The most aggressive type of brain cancer is glioblastoma (GBM), which has a high cellular heterogeneity. This complexity is connected to treatment resistance and a poor prognosis (Louis et al., 2016).
- Traditional bulk RNA sequencing generates an average gene expression profile, concealing the variety of the tumor microenvironment (Patel et al., 2014).
- Single-cell RNA sequencing (scRNA-seq) provides unparalleled resolution, allowing researchers to identify and characterize specific GBM cell populations (Cuperus, 2022).
- This study uses scRNA-seq data and Seurat analysis to fulfil three major objectives:
  - Discover the cellular heterogeneity of GBM. We intend to find and classify various subpopulations inside the tumor by clustering cells based on gene expression features.
  - Identify possible therapeutic targets. Each subpopulation may exhibit distinct markers, creating prospects for targeted therapy.
  - Gain understanding about GBM biology: Characterizing cell types and their interactions can provide insight into carcinogenesis, progression, and potential treatment vulnerabilities.

2. Methods

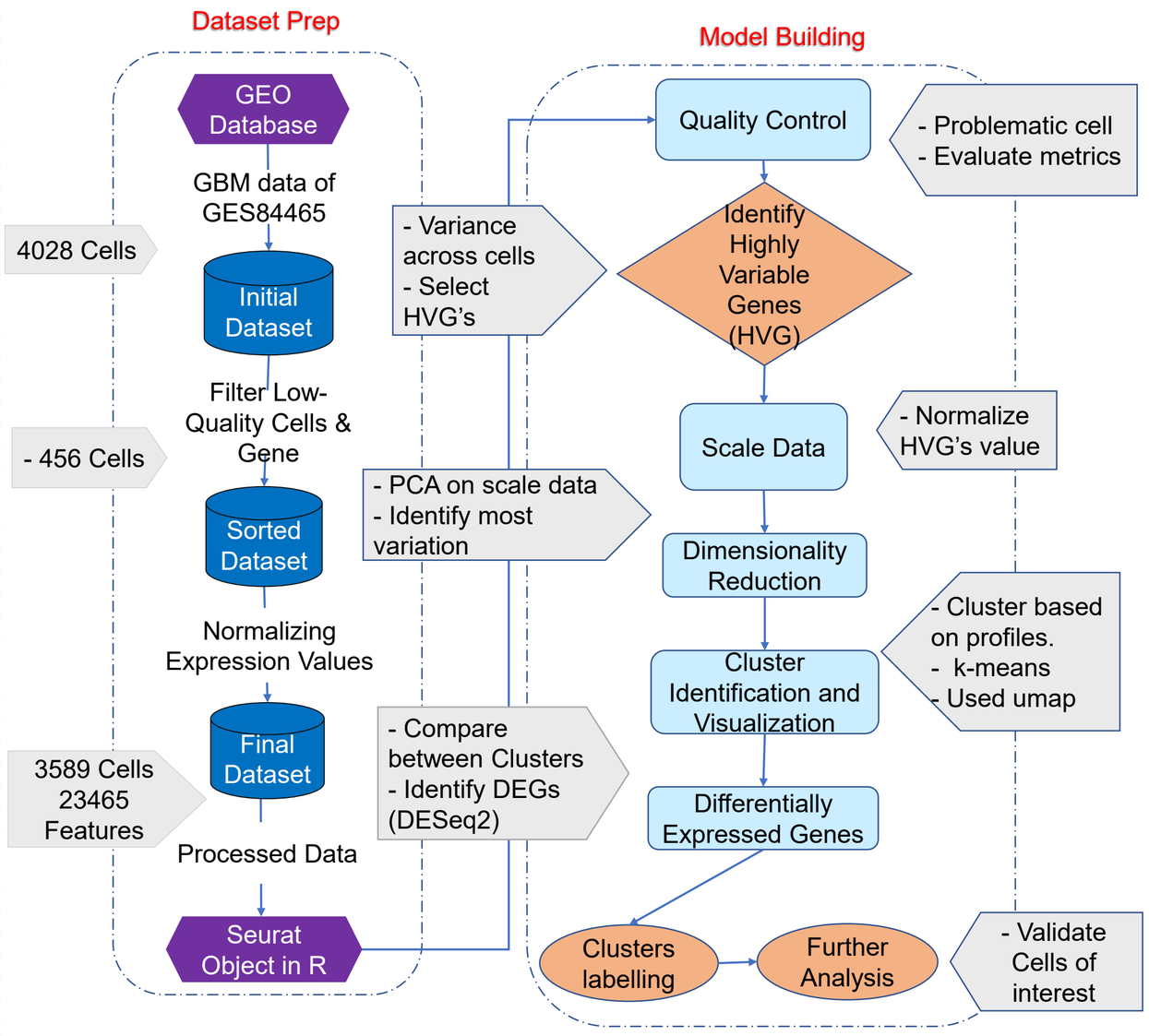

Fig.1. Single-cell RNA Sequencing Data Analysis in R: From Initial Dataset Prep to Identifying Differentially Expressed Genes and Clusters

- The Fig.1. illustrates the workflow of the data analysis conducted in R Studio version 2022.12.0. The analysis implemented the Seurat 5.0.2 library in model to identify differentially expressed genes and clusters on scRNA data.

3. Results

- The clusters are colored based on their assigned labels, determined using the FindClusters function in Seurat_R, providing a visual representation of the cell populations and their distribution within the tumor.
- Table 1. lists the top 2 genes in each population, ranked by their log2 fold change, p-value, and adjusted p-value.
- The UMAP plot in Fig. 2 shows the classified subpopulations inside the tumor based on expression, revealing 7 clusters of cells.

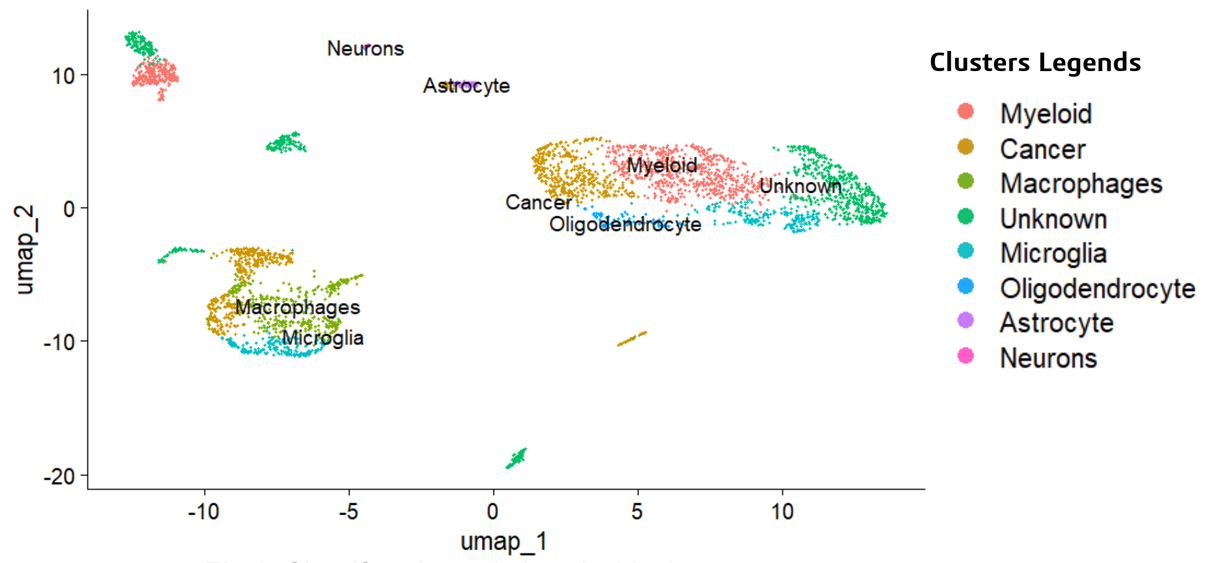

Fig.2. Classify subpopulations inside the tumour based on expression.

Table 1. Top 2 Genes in each Cluster.

Cluster	Gene	log2FC	p_val	p_val_ad
Myeloid	NKAIN4	3.314448661	1.03E-57	2.42E-53
Myeloid	CSPG4	2.988907497	1.10E-36	2.57E-32
Cancer	ANPEP	3.736621744	2.99E-109	7.01E-105
Cancer	MARCO	4.006478341	7.44E-97	1.75E-92
Macrophages	C10orf10	2.798343319	2.00E-48	4.69E-44
Macrophages	SPEG	3.085244339	6.44E-30	1.51E-25
Unknown	DHRS9	3.855360575	3.24E-127	7.60E-123
Unknown	IPCEF1	3.402968982	1.49E-125	3.50E-121
Microglia	CA9	5.241558818	2.70E-103	6.33E-99
Microglia	LOX	4.953645346	1.09E-77	2.56E-73
Oligodendrocyte	AURKB	5.487193424	0	0
Oligodendrocyte	CEP55	5.706045792	1.07E-251	2.50E-247
Astrocyte	GJB6	10.25934403	0	0
Astrocyte	WIF1	9.437424743	0	0
Neurons	GAD2	13.0063696	0	0
Neurons	VIP	13.25702774	7.87E-244	1.85E-239

4. Discussion

- The study used single-cell RNA sequencing data of glioblastoma (GBM) and the Seurat package in R (Hao et al., 2024) to classify subpopulations inside the tumor based on expression using UMAP.
- Each subpopulation may exhibit distinct markers, creating prospects for targeted therapy.
- Table 1. provides information on the most significantly differentially expressed genes in each population, which can be used to identify potential therapeutic targets.
- GBM Biology: example: CSPG4 gene from the myeloid cluster can be involved in the tumor micro-environment by influencing the interaction between tumor cells and the extracellular matrix, which is crucial for tumor growth and metastasis (Campoli et al., 2004).

5. Conclusions

- The findings of this study can be applied to expand knowledge on the cellular heterogeneity of GBM and the development of targeted therapies
- Future studies can focus on validating the identified markers and their potential as therapeutic targets.

6. References

- LOUIS, D. N., PERRY, A., REIFENBERGER, G., VON DEIMLING, A., FIGARELLA-BRANGER, D., CAVENEE, W. K., OHGAKI, H., WIESTLER, O. D., KLEIHUES, P. & ELLISON, D. W. 2016. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol,* 131**,** 803-20.
- PATEL, A. P., TIROSH, I., TROMBETTA, J. J., SHALEK, A. K., GILLESPIE, S. M., WAKIMOTO, H., CAHILL, D. P., NAHED, B. V., CURRY, W. T., MARTUZA, R. L., LOUIS, D. N., ROZENBLATT-ROSEN, O., SUVÀ, M. L., REGEV, A. & BERNSTEIN, B. E. 2014. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science,* 344**,** 1396-401.
- CUPERUS, J. T. 2022. Single-cell genomics in plants: current state, future directions, and hurdles to overcome. *Plant Physiol,* 188**,** 749-755.
- HAO, Y., STUART, T., KOWALSKI, M. H., CHOUDHARY, S., HOFFMAN, P., HARTMAN, A., SRIVASTAVA, A., MOLLA, G., MADAD, S., FERNANDEZ-GRANDA, C. & SATIJA, R. 2024. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nature Biotechnology,* 42**,** 293-304.
- CAMPOLI, M. R., CHANG, C. C., KAGESHITA, T., WANG, X., MCCARTHY, J. B. & FERRONE, S. 2004. Human high molecular weight-melanoma-associated antigen (HMW-MAA): a melanoma cell surface chondroitin sulfate proteoglycan (MSCP) with biological and clinical significance. *Crit Rev Immunol,* 24**,** 267-96.