Analysis of Differential Gene Expression and Pathway Alterations in Glioblastoma comparing with Healthy Cell

Introduction

This study analyzes spatial transcriptomics data from 43 GB tumors and their matched healthy tissues (HC), totaling 86 samples. Using the GeoMx platform, we investigate gene expression differences and classify tumors: GB_1 (metastatic, poor prognosis) and GB_2 (non-metastatic, better prognosis).

Principal Component and Survival Analysis

Principal Component Analysis (PCA) (Figure 1A) reveals a clear separation between HC, GB_1, and GB_2, indicating significant differences in gene expression. The Survival curve (Figure 1B) shows that GB_1 has a significantly shorter survival time than GB_2 ($p = 0.0076$). This suggests that GB_1 tumors are more aggressive and associated with worse clinical outcomes.

A vs B Analysis

1. Differential Gene Expression Analysis

Figures 1C and 1D (MA and volcano plots) highlight differentially expressed genes. Figure 2A presents the top ten genes with significant expression changes.

- **GB_1 vs. HC (**Figure 2A (i)**)**: **TSPAN13**, a Tetraspanin family member, is significantly upregulated in GB1. Tetraspanin family proteins are highly associated with GB. For instance, CD151 is frequently overexpressed in GB, promoting stemness, self-renewal, growth, and migration. Inhibiting CD151 has been suggested as a potential GB treatment strategy (Tilghman et al., 2016). Given their shared family, **TSPAN13** may similarly promote GB growth. Studies also report **that TSPAN13** has significantly increased in GB patients, especially those with **temozolomide (TMZ) resistance**, which is a drug for treating Glioblastoma, suggesting its potential as a prognostic

biomarker (Wang et al., 2025). Another notable gene, **CDH1**, is significantly upregulated in both GB1 and GB2. CDH1 is a tumor suppressor, and its **methylation may contribute to GB progression (Belut et al., 2024)**. Its upregulation could be a result of negative feedback mechanisms.

- **GB_2 vs. HC (**Figure 2A (ii)**)**: LAMB3 is significantly upregulated, particularly in GB_2. LAMB3, a component of laminin 5, plays a role in cell proliferation, migration, and the cell cycle (Yu et al., 2024). Although no direct studies link LAMB3 to GB, further investigation is necessary. Another gene, **SDC4**, is significantly upregulated in both GB_1 and GB_2. **SDC4** is implicated in cancer pathogenesis and may serve as a **therapeutic target** (Onyeisi et al., 2021). Enrichment pathway analysis (Figure 2B (i) and Figure 2B (ii)) both link to the extracellular matrix. Remodeling the extracellular matrix (ECM) will support Glioblastoma growth and invasion. Tumor cells secrete enzymes like matrix metalloproteinases (MMPs) to break down the ECM, creating space for migration (Furnari et al., 2007). These ECM changes also affect cell adhesion and signaling, further promoting tumor progression (Gritsenko et al., 2012)
- **GB_1 vs. GB_2 (**Figure 2A (iii)**)**: HNRNPA2B1 is significantly upregulated in GB_1. This gene regulates VEGFR2, which plays a crucial role in vasculogenesis, potentially contributing to tumor vascularization (Wang et al., 2024).

2. Pathway Enrichment Analysis

Enrichment analysis (Figures 2B (i) and (ii)) shows that differentially expressed genes link to **extracellular matrix organization** pathway. Tumor cells secrete enzymes like matrix metalloproteinases (MMPs) to break down the ECM, supporting migration and invasion (Furnari et al., 2007). ECM changes also affect cell adhesion and signaling, further promoting tumor progression (Gritsenko et al., 2012).

Enrichment analysis in Figure 2B (iii) links to **Tissue and Epithelial Homeostasis** might reduce epithelial-to-mesenchymal transition (EMT), a key process in GBM aggressiveness and metastasis (Lee et al., 2014).

[A vs B]n Analysis

1. Correlation and Overlap Analysis

Figure 3A (i) shows a moderate negative correlation ($r = -0.564$, $p < 2.2e-16$) between GB_1 vs. HC and GB_1 vs. GB_2, suggesting that upregulated genes in one condition tend to be downregulated in the other. Figure 3A (ii) shows a weak but significant positive correlation ($r = 0.171$, $p < 2.2e-16$) between GB_2 vs. HC and GB_1 vs. GB_2, indicating shared but context-dependent gene regulation. Venn diagram analysis (Figure 3B) reveals a significant overlap in differentially expressed genes, suggesting common biological mechanisms.

2. Expression Trends in Key Genes and Pathway Enrichment Analysis

Figures 3C shows a **heatmap** of significant overlap genes from Figure 3B. Two major expression trends emerge:

- **HC-high, GB_1-low, GB_2-increasing (figures 3C (i)):** Including TRBC1, IL2RG, MXRA5, LYZ, and POU2AF. MXRA5, in particular, is associated with PTEN-dependent GBM survival (Sai Krishna et al., 2025). IL-2R is the most relevant gene in mature B cell differentiation, and it is the most significant pathway analysis by Pathway Enrichment Analysis (Figure 4A (i)). However, its role in cancer is dual-faceted, promoting anti-tumor immunity and immune suppression via Tregs, making it a key regulator of the tumor microenvironment rather than a typical oncogene (Shouse et al., 2024). Further research is needed to clarify its precise function.
- **HC-low, GB_1-high, GB_2-decreasing:** Enrichment analysis (Figure 4A (ii)) associates these genes with **multicellular organismal level chemical homeostasis**. Genes such as LPCAT1, CKB, and AQP3 link to tumor progression. LPCAT1 regulates lipid metabolism and remodeling (Bi et al., 2019), CKB influences creatine metabolism (Katz et al., 2024), and AQP3 is Aquaporins, also a prognostic marker in GBM (Varricchio & Yool, 2023).
- **GB_2-specific:** EFEMP1 is downregulated in GB_2, potentially increasing sensitivity to temozolomide (TMZ) (Hiddingh et al., 2014). Upregulated genes (e.g., ADAM28, ANXA10, LGALS4) are associated with **epithelial structure**

maintenance pathway (figure 3D (iii)), which may reduce EMT and GBM aggressiveness (Lee et al., 2014).

Conclusion

This analysis highlights distinct gene expression patterns between GB_1, GB_2, and HC, revealing potential biomarkers and therapeutic targets. TSPAN13, LAMB3, and SDC4 are upregulated in GBM and may contribute to tumor progression. In contrast, MXRA5 and EFEMP1 link to prognostic outcomes. Additionally, genes involved in multicellular homeostasis and EMT regulation could influence GB aggressiveness. While this study identifies key molecular signatures, several differentially expressed genes and pathways remain unexplored due to limited available literature. Further functional validation and mechanistic studies are required to elucidate their roles in GBM pathology and assess their potential as therapeutic targets.

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