Integrated Metabolomics and Transcriptomics Analysis of Monolayer and Neurospheres from Established Glioblastoma Cell Lines

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Introduction

Glioblastomas are very aggressive tumours without efficient treatment, where cancer stem-like cells are thought to be responsible for relapse. This pilot study investigated the metabolic discrepancies between monolayer and neurosphere cultures of two glioblastoma cell lines using transcriptomics and metabolomics. We show that the two culture systems display substantial differences regarding their metabolome and transcriptome. Specifically, we found that metabolic reactions connected to arginine biosynthesis are crucial to support the different metabolic needs of neurospheres from the two cell lines. By identifying metabolic vulnerabilities in different glioblastoma subpopulations, new therapeutic strategies may be emerging that can be explored to treat this disease. Moreover, this data set may be of great value as a resource for the scientific community.

Materials and Methods

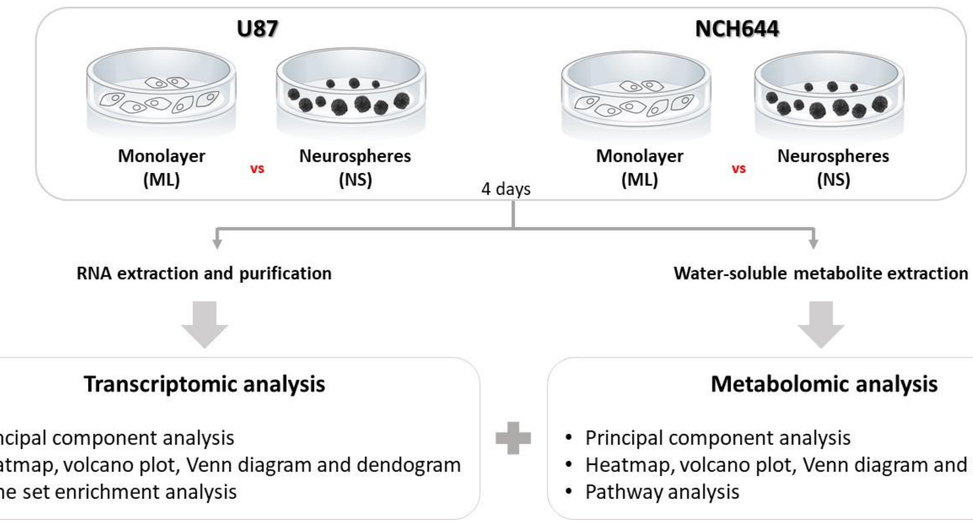

Figure 1 – Culture of two GB cell lines (U87 and NCH644) in adherent conditions with serum-containing medium (monolayer – ML) and non-adherent conditions with serum-free medium to select for stem-like cells (neurospheres – NS). RNA and water-soluble metabolites were extracted from these conditions to perform transcriptomic and metabolomic analysis, respectively. Finally, joint pathway analysis was performed in MetaboAnalyst to reveal the most signficantly regulated metabolic pathways in NS vs ML cells.

Results

Transcriptomic profile

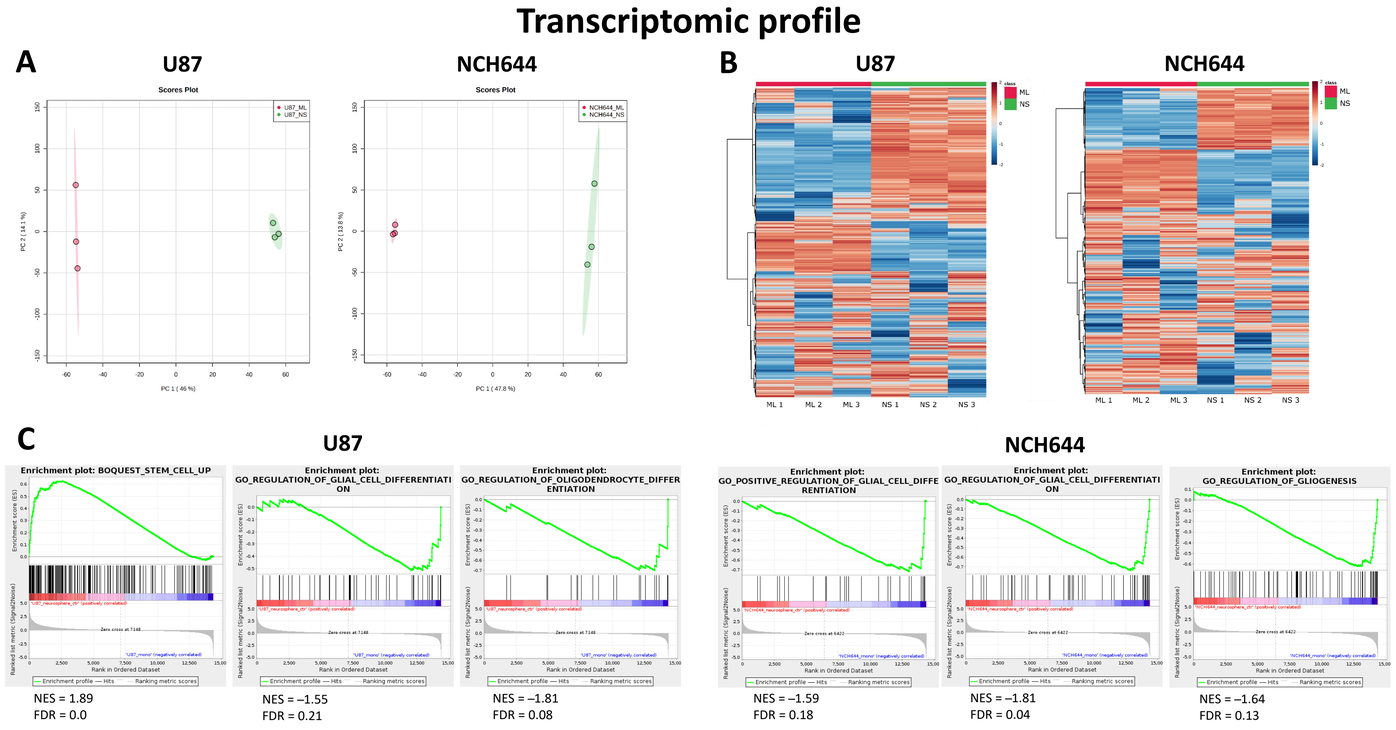

Figure 2 – Transcriptomics analysis of monolayer (ML) and neurospheres (NS) from U87 and NCH644 cells. (A) Principal component analysis of all genes showing a clear separation between ML and NS samples in both cell lines. **(B)** Heatmaps showing upregulated (red) and downregulated (blue) genes in NS vs. ML from U87 and NCH644 cells (FDR < 0.05, log2FC > ±1). **(C)** Gene set enrichment analysis showing the upregulation of a stem-related gene signature and the downregulation of differentiation gene signatures in NS vs. ML from U87 and NCH644 cells (FDR < 0.25). n = 3

Metabolomic profile

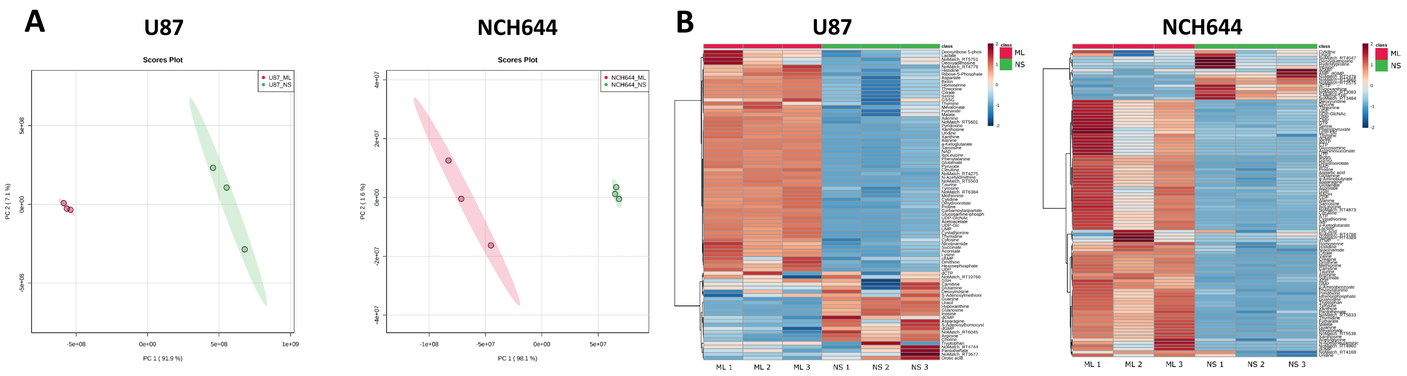

Figure 3 – Metabolomics analysis of ML and NS from U87 and NCH644 cells. (A) Principal component analysis of all metabolites showing a clear separation between ML and NS samples in both cell lines. **(B)** Heatmaps of increased (red) and decreased (blue) metabolite levels in NS vs. ML from U87 and NCH644 cells (FDR < 0.05). n = 3

Joint pathway analysis

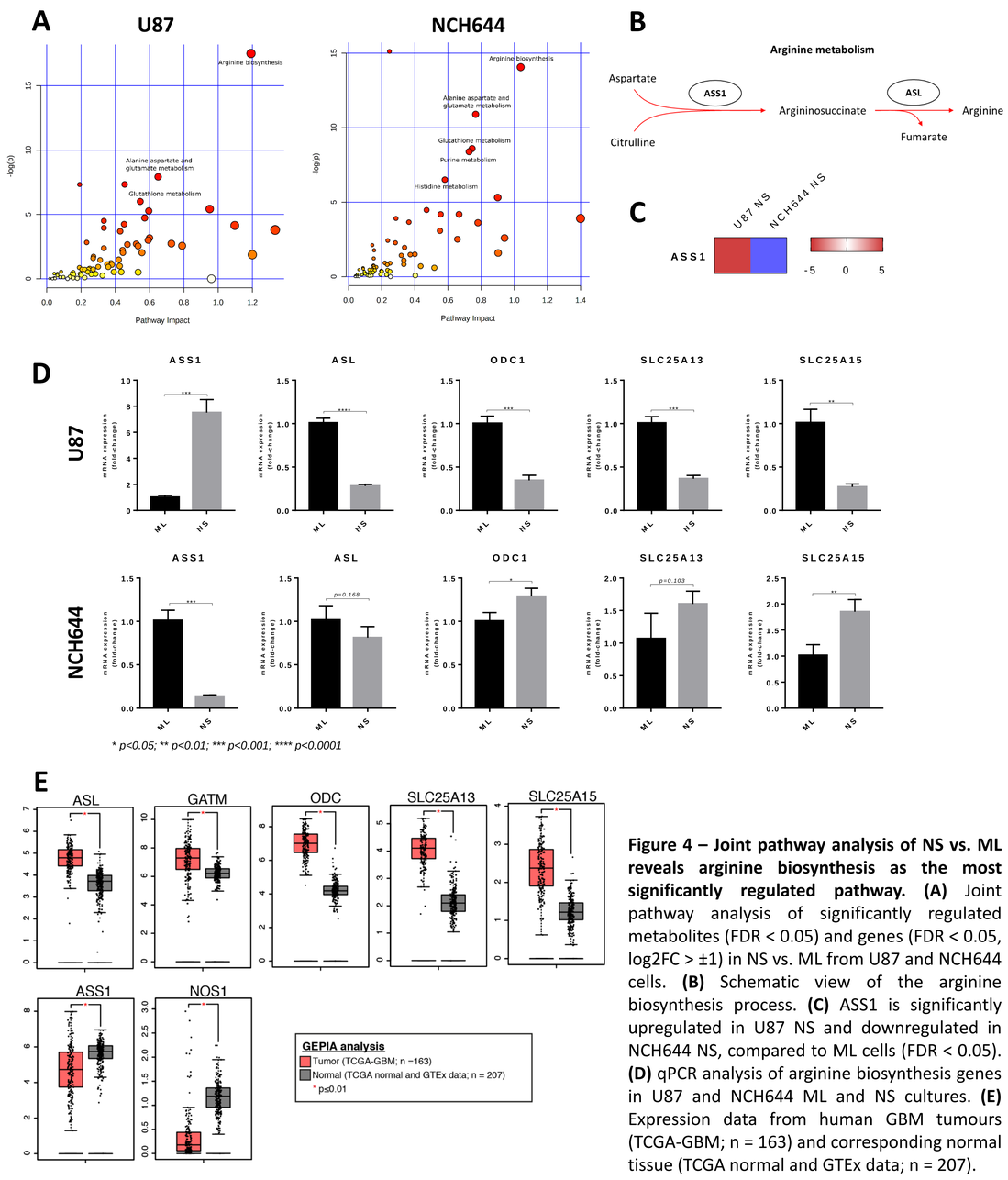

Figure 4 – Joint pathway analysis of NS vs. ML reveals arginine biosynthesis as the most significantly regulated pathway. (A) Joint pathway analysis of significantly regulated metabolites (FDR < 0.05) and genes (FDR < 0.05, log2FC > ±1) in NS vs. ML from U87 and NCH644 cells. **(B)** Schematic view of the arginine biosynthesis process. **(C)** ASS1 is significantly upregulated in U87 NS and downregulated in NCH644 NS, compared to ML cells (FDR < 0.05). **(D)** qPCR analysis of arginine biosynthesis genes in U87 and NCH644 ML and NS cultures. **(E)** Expression data from human GBM tumours (TCGA-GBM; n = 163) and corresponding normal tissue (TCGA normal and GTEx data; n = 207).

Discussion

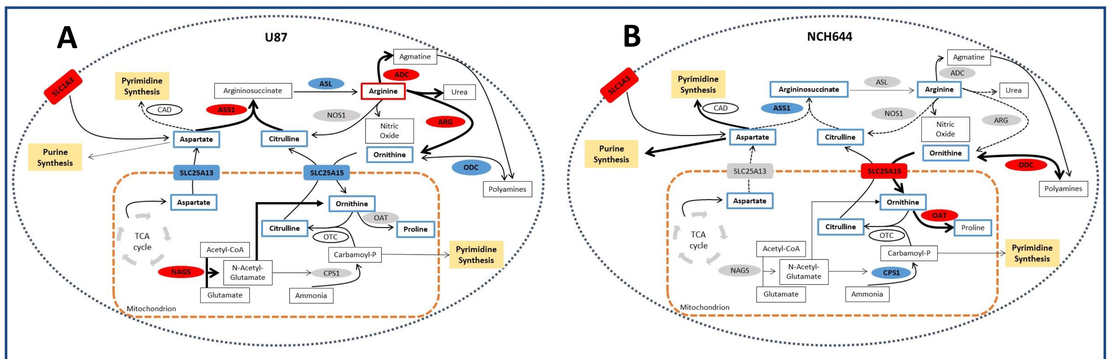

Figure 5 – Proposed model of arginine metabolism in U87 and NCH644 neurospheres. (A) U87 NS present ASS1 upregulation, pointing towards aspartate shuttling into arginine synthesis and a reduction of nucleotide metabolism. **(B)** NCH644 NS, by downregulating ASS1, may shuttle aspartate away from arginine biosynthesis and towards nucleotide production.

Conclusions

- The two cell lines were able to adapt to the different culture conditions—monolayer cultures that induce a more differentiated state and neurosphere cultures that select for stem-like cells;
- The metabolic portrait of GSCs is fundamentally different from that of cells in a more differentiated state;
- Arginine biosynthesis may be a key metabolic pathway for the regulation of stem-like features in GBM;
- The regulation of individual nodes within arginine biosynthesis pathway in the two cell lines resulted in different metabolic outcomes, to achieve specific metabolic demands of each cell line;
- Several enzymes involved in arginine biosynthesis, including ASS1, were also found to be significantly regulated in human GBM, suggesting that NCH644 cells may more closely resemble the in vivo setting of this disease.

Bibliography

- Peixoto J, *et al.* (2021) Integrated Metabolomics and Transcriptomics Analysis of Monolayer and Neurospheres from Established Glioblastoma Cell Lines. Cancers
- Peixoto J, Lima J (2018) Metabolic traits of cancer stem cells. DMM

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