circRNA in glioblastoma - preliminary analysis

International Doctoral School
Medical University of Lodz

PhD Student: Bartosz Lenda, MS
Assistant supervisor: Marta Żebrowska-Nawrocka, PhD
Supervisor: prof. Ewa Balcerczak, PhD
Department of Pharmaceutical Biochemistry and Molecular Diagnostics

Introduction

This work is a part of a doctoral thesis entitled *Evaluation of the expression of selected circRNA molecules and their clinical potential as liquid biopsy biomarkers in patients with brain tumors*. Although circulating biomarkers possess a great clinical potential in terms of improving patients' diagnosis and prognostication, it is still important to deepen the understanding of brain tumors' molecular pathogenesis, including glioblastoma (GBM), WHO grade 4 tumor. Besides, some of the serum exosomal non-coding transcripts exert inverse expression as compared to tumor tissue. Thus, as a first stage of the project, we decided to focus on GBM tissue specific circRNA and their interactions in ceRNA networks. For this purpose, we conducted differential expression analysis of publicly available microarray dataset. The aim of this poster is to present preliminary results of bioinformatics analysis as well as laboratory protocol developed for experimental validation of *in silico* studies.

Materials and methods

To perform *in silico* analysis, we obtained Gene Expression Omnibus (GEO) dataset GSE165926. Study design of this dataset is presented in Table 1. For further studies, we included brain tissue samples only from patients diagnosed with glioblastoma WHO G4 and cortical brain samples as a control group. Arraystar names of circular transcripts were converted to circBase IDs with CircPrimer 2.0 online tool. Then, we utilized R packages *GEOquery*, *dplyr* and *limma* to perform differential expression (DE) analysis. For data integrity check, we generated PCA plot and heatmap (R packages *ggplot2* and *pheatmap* respectively). For volcano plot, we used R package *ggplot2*.

To establish interactions within the ceRNA networks, additional GEO dataset GSE4290 was obtained and analyzed with online tool GEO2R. Pairs of DEcircRNA-miRNA and miRNA-DEmRNA were downloaded from CircBank and miRTarBase respectively and then intersected for competing endogenous RNA networks in GBM patients. Detailed workflow is presented in Figure 1.

To perform Gene Ontology enrichment analysis for protein-coding genes in ceRNA networks, R packages *clusterProfiler* and *org.Hs.eg.db* were utilized. All analyses were performed in RStudio 2023.12.1. software.

To further verify results of *in silico* analysis, we developed qRT-PCR protocol (Figure 2.). Divergent primers for circular transcripts were designed according to the instruction by (Panda & Gorospe, 2018) and verification of their specificity was conducted in CircPrimer2.0 software (Figure 3.). 10 glioblastoma tissue samples and 10 paired brain tissue samples from the resection margin were collected in the Bródnowski Masovian Hospital, Warsaw during a surgical procedure. The consent of the Bioethics Committee was obtained to conduct the study.

GEO ID	Platform	Study design			
		Tumor tissue			Control tissue
		WHO G2	WHO G3	WHO G4	
GSE165926	074301 Arraystar Human CircRNA microarray V2	ganglioglioma (1)	anaplastic astrocytoma (1)	**glioblastoma multiforme (5)**	**cortical brain tissue (4)**
		oligodendroglioma (1)	anaplastic oligodendroglioma (2)		
		diffuse astrocytoma (1)			
		anaplastic glioma (1)			

Table 1. Study design of GSE165926 dataset, which consists of 16 tissue samples. Number of samples in parentheses.

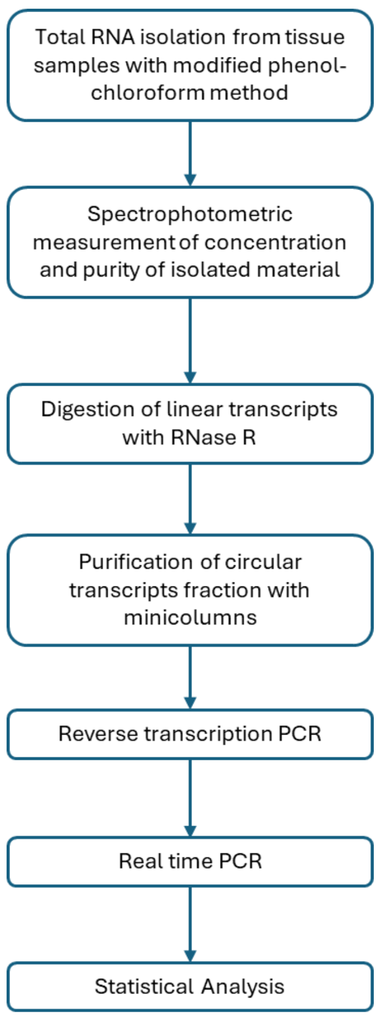

Figure 2. qRT-PCR protocol for circRNA expression study.

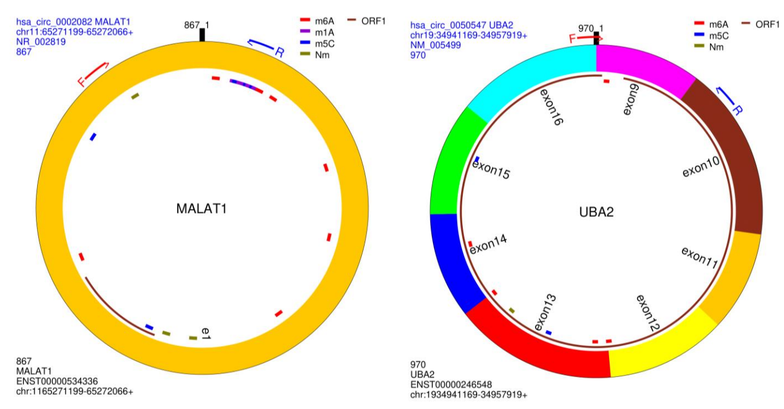

Figure 3. Divergent primers for two circRNAs; *hsa_circMALAT1_024* (left) and *hsa_circUBA2_052* (right).

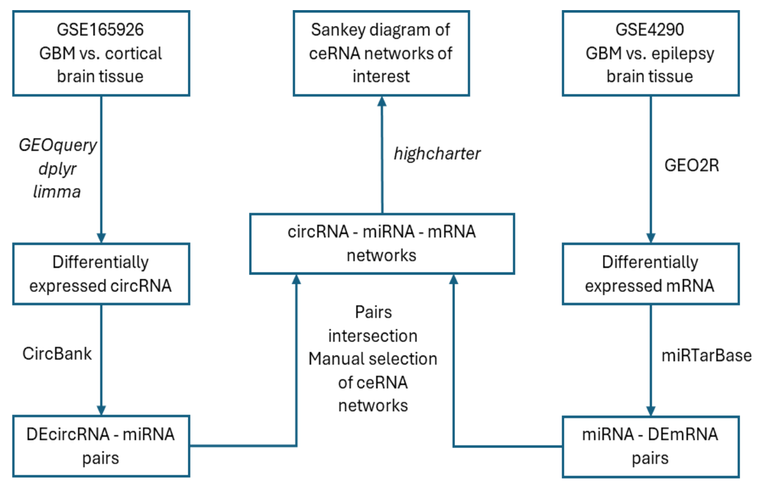

Figure 1. Workflow of bioinformatics analysis to obtain ceRNA networks from GSE165926 and GSE4290 datasets.

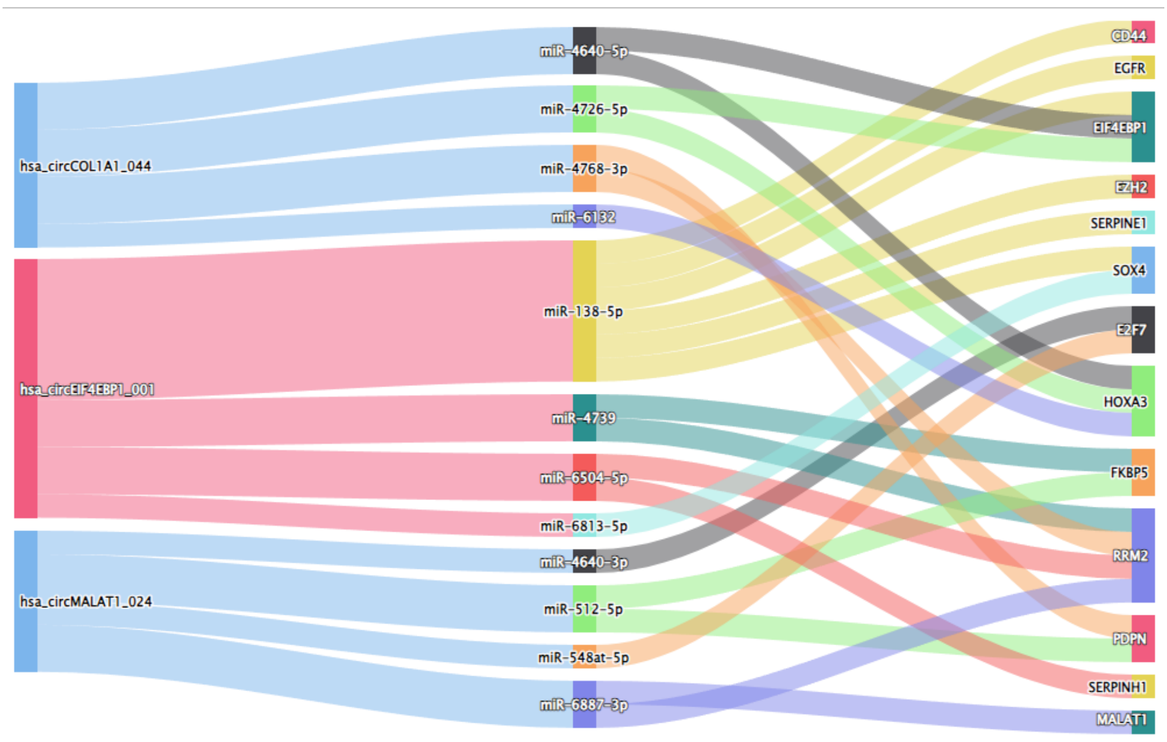

Figure 5. Sankey diagram for potential circRNA-specific ceRNA networks in GBM patients.

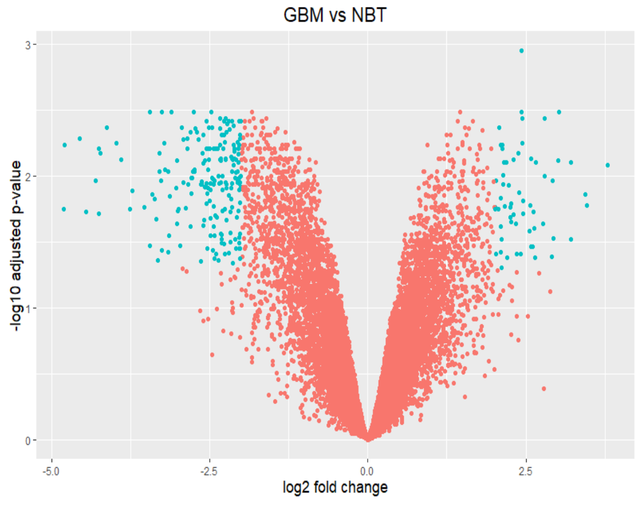

Figure 4. Volcano plot for differentially expressed circRNA in GBM patients. Significant up- and downregulated genes are considered with thresholds |log2FC|>2 and padj<0,05 (blue points). GBM- glioblastoma; NBT- normal brain tissue (cortical brain tissue).

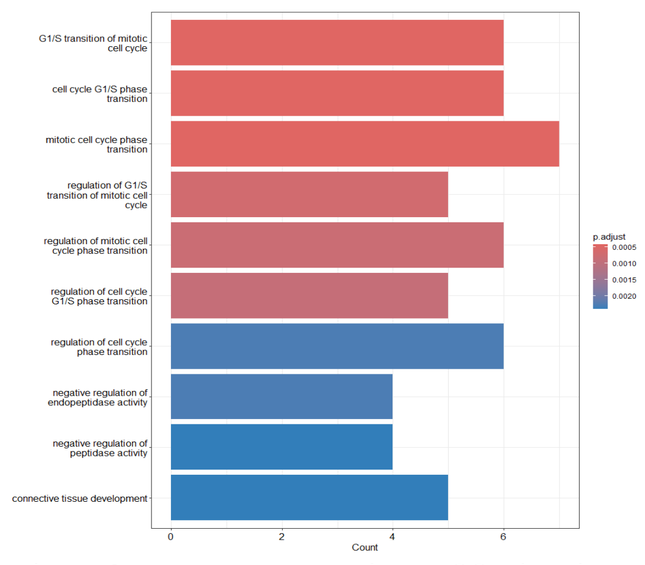

Figure 6. Top ten most enriched GO biological processes of protein coding genes in circRNA-specific ceRNA networks in GBM patients.

Preliminary results and future perspective

Differential expression analysis showed that 60 circRNA were significantly top upregulated and 194 circRNA were significantly top downregulated in analyzed dataset (|log2FC|>2, padj<0,05; Figure 4.). Based on these results and a literature review, we selected three circRNAs for further laboratory validation: *hsa_circMALAT1_024*, *hsa_circUBA2_052* and *hsa_circVPS8_032*.

Interestingly, the parental gene *MALAT1* belonging to the class of long non-coding RNA, was the subject of our review paper, in which the collected literature indicated its association with an epithelial to mesenchymal transition activating family of ZEB transcription factors in tumors of glial origin (Lenda et al., 2023). As a result of ceRNA network analysis, we noticed that *MALAT1* may be the downstream target of its circular variant via interacting with *miR-6887-3p* in a feedback loop manner (Figure 5.). Circular transcripts belonging to the *MALAT1* family may thus represent promising prospects for the understanding of pathogenesis of human gliomas at the molecular level.

Gene Ontology enrichment analysis showed an interesting trend, with the most enriched GO biological processes being mainly connected with cell cycle regulation, especially G1/S transition (Figure 6.). This can be valuable information for planning further studies on cell biological functions in GBM cell lines.

The results presented in this work need an experimental verification, which is planned for the rest of an academic year. As of a date of preparing this work, 20 samples of total RNA from GBM patients were isolated and further steps of the protocol are ongoing. In a near future, we also want to broaden the scope of our interest to the topic of serum specific circRNA and other non-coding transcripts.

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