

### Introduction

Glioblastoma multiforme (GBM) is the most often occurring intracranial malignancy of astrocyte origin in adults. This tumor disease is characterized by infaust prognosis, which is primarily caused by resistance to the therapy and early relapses relate to the presence of glioblastoma stem cells (GSCs). Targeting of GSCs could be a novel promising therapeutic approach leading to the overcome of therapy resistance and better prognosis of GBM patients. One of the approaches how to successfully regulate GSC is a targeted regulation of microRNAs (miRNAs). These small non-coding RNA molecules post-transcriptionally regulate an expression of more than 2/3 of all human genes that are also involved in stem cell associated signaling pathways.

### Methods

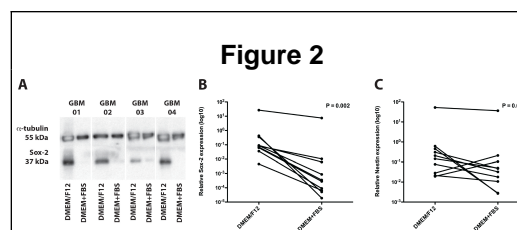
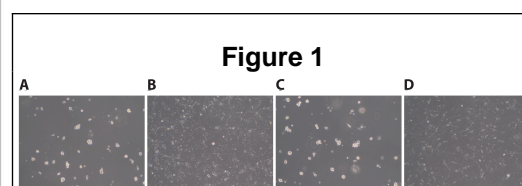
We have prepared ten Sox-2 high- and low-expressing paired primary GBM cell lines, which have been cultured under non-serum and serum conditions, respectively. The global miRNA expression analysis was performed using GeneChip miRNA 4.0 Array (Affymetrix). Sox-2 and nestin expressions were analyzed on both protein and transcriptional levels using combination of PAGE with Western blotting and real-time PCR, respectively. Targeted regulation of miRNA levels have been carried out by the transient transfection of specific anti-miRNAs or miRNA mimics in GSC cell lines NCH 601 acquired from Interdisciplinary Center For Neurosciences (Heidelberg, Germany).

### Results

Analyze of Sox-2 positive and negative paired GBM cell lines revealed 29 differentially expressed miRNAs, from which miR-93-3p, miR-95-5p, miR-106b-5p, miR-22-3p, and miR-3195 showed high significance (adjust. P value < 0.05) and association with both Sox-2 (Spearman R;  $p < 0.002$ ) and nestin (Spearman R;  $p < 0.02$ ) expressions. MiR-22-3p and miR-3195 showed lower whereas other miRNAs higher expression in Sox-2 positive GBM cells.

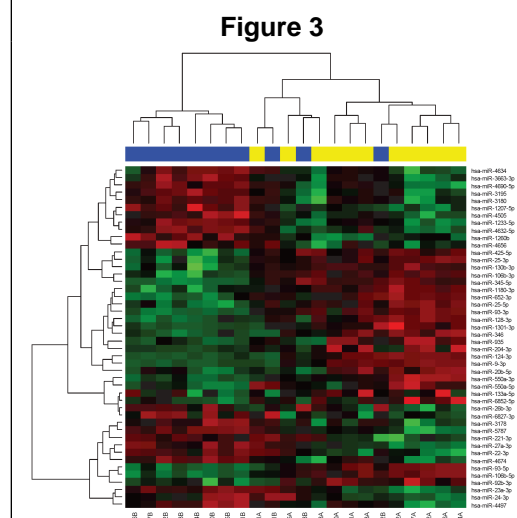
### Conclusions

Our data suggest that these miRNAs are probably involved in GSC biology maintaining and, thus, are analyzed in vitro to be promising molecular targets to overcome GBM therapeutic resistance at this time. Results of in vitro analyses will be presented. This work was supported by grants of Internal Grant Agency of the Czech Ministry of Health no. NT13514-4/2012, 15-33158A; and project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068).



Characterization of the primary GBM cell lines propagated in serum-free and serum containing media. A - western blot analysis of Sox-2 and -tubulin and qRT-PCR of B - Sox-2 and C - expression in GBM cells propagated in serum-free (DMEM/F12) and serum containing (DMEM+FBS) media.

The p value signifies the statistical significance of the difference between the paired primary cell lines as assessed by the Wilcoxon paired test.



Hierarchical clustergram discriminating Sox-2 high (yellow) and Sox-2 low (blue) expressing paired primary GBM cell lines propagated in serum-free and serum containing media, respectively. Based on 51 differentially expressed miRNAs (with a  $P < 0.001$ ).

Table 1

miRNA	Sox-2		Nestin	
	Spearman r	P value	Spearman r	P value
miR-3195	-0,85	< 10-5	-0,60	0,006
miR-3141	-0,83	< 10-5	-0,47	0,036
miR-4656	-0,81	< 10-4	-0,51	0,023
miR-100-5p	-0,79	< 10-4	-0,39	NS
miR-4739	-0,77	< 10-3	-0,42	NS
miR-3180	-0,75	< 10-3	-0,55	0,013
miR-1260b	-0,75	< 10-3	-0,46	0,043
miR-1233-5p	-0,74	< 10-3	-0,49	0,029
miR-4674	-0,73	< 10-3	-0,54	0,015
miR-328-5p	-0,73	< 10-3	-0,48	0,032
miR-378h	-0,72	< 10-3	-0,48	0,034
miR-4505	-0,71	< 10-3	-0,46	0,045
miR-5787	-0,71	< 10-3	-0,47	0,036
miR-1207-5p	-0,70	< 10-3	-0,37	NS
miR-345-5p	0,82	< 10-5	0,57	0,011
miR-1180-3p	0,78	< 10-4	0,45	0,048
miR-9-3p	0,76	< 10-3	0,40	NS
miR-124-3p	0,75	< 10-3	0,34	NS
miR-106b-3p	0,73	< 10-3	0,42	NS
miR-1301-3p	0,73	< 10-3	0,41	NS
miR-130b-3p	0,71	< 10-3	0,49	0,029
miR-93-3p	0,70	< 10-3	0,37	NS
miR-106b-5p	0,70	< 10-3	0,32	NS

Spearman correlation of miRNAs with Sox-2 and nestin expression in paired primary GBM cell lines.

### Learning Objectives

By the conclusion of this session, participants should be able to understand a new possibility of targeting glioblastoma stem cells.

### References

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