Hypoxia is a critical aspect of the glioma microenvironment and has been associated with poor prognosis and resistance to various therapies. However, the mechanisms responsible for hypoxic survival of glioma cells remain unclear. Recent studies strongly suggest that microRNAs act as critical mediators of the hypoxic response. We thus hypothesized their prominent role in hypoxia resistance in glioblastoma (GBM) and aimed to identify those.

Here, we present the first detailed analysis of small RNA transcriptome of cell line U87MG, a grade IV glioma cell line, and its alteration under hypoxic condition. Based on deep sequencing and microarray data, we identify a set of hypoxia regulated microRNAs, a subset of which was validated in GBM cell lines U87MG and U251MG by stem-loop qRT-PCR. We show miR-210, miR-1275, miR-376c-3p, miR-23b-3p, miR-193a-3p and miR-145-5p to be up-regulated, while miR-92b-3p, miR-20a-5p, miR-10b-5p, miR-181a-2-3p and miR-185-5p are down-regulated by hypoxia. Notably, miR-210 and its isomiRs showed highest induction under hypoxia. Interestingly, certain hypoxia-induced miRNAs are also known to be over-expressed in GBM tumors, suggesting that hypoxia may be one of the factors involved in establishing the miRNA signature of GBM. Transcription factor binding sites for Hypoxia inducible factor 1 A (HIF1A) were identified in the promoter region (5 kb upstream) of 30 hypoxia-induced miRNAs. HIF-1A over-expression and silencing studies show regulation of specific miRNAs, including miR-210, to be HIF1A dependent. On the other hand, miR-210 leads to an increase in transcriptional activity of HIF and its target genes vascular endothelial growth factor (VEGF) and carbonic anhydrase 9 (CA9). MiR-210 levels were found to be high in astrocytic patient samples in a grade dependent manner and showed good correlation with the known hypoxia markers CA9 and VEGF. We show that miR-210 promotes cell proliferation, migration, hypoxic survival, chemoresistance and inhibits apoptosis in GBM cells and targets a negative regulator of hypoxic response, HIF3A and
a neuronal differentiation factor, NeuroD2. Our analyses of the TCGA-GBM data revealed significant downregulation of NeuroD2 in GBM patients. Low levels of NeuroD2 were found to be correlated with poor overall survival of GBM patients. NeuroD2 was shown to be transcriptionally induced by p53. NeuroD2 overexpression diminished GBM aggressiveness by inhibiting cell proliferation, migration and promoting apoptosis under hypoxia. NeuroD2 overexpressing glioma cells failed to form 3D-tumor spheroids and displayed reduced migration in a 3D gelatin matrix. NeuroD2 gene-signature was enriched in pathways belonging to cytokine-cytokine receptor interaction, TNF-signaling, PI3K-AKT signaling, focal adhesion and ECM-receptor interaction. Moreover, miR-210 mediated above mentioned multiple hallmarks of cancer were partly dependent on the targeted downregulation of NeuroD2.

Additionally, a total of 139 novel miRNAs were discovered by the analysis of deep sequencing data. Five novel miRNAs were validated by qRT-PCR, and three of these (iithsa_40, iithsa_15 and iithsa_92) were found to be differentially expressed under hypoxia. The regulation and the functional role of these novel miRNAs under hypoxic conditions remains to be seen.

Overall, our study reveals a novel miRNA signature of hypoxia in GBM and suggests miR-210 to be an oncogenic player and a novel potential intrinsic marker of hypoxia in GBM. Furthermore, our study identifies a novel role of NeuroD2 as a tumor suppressor in GBM the levels of which are tightly regulated by p53 and miR-210. Overexpressing NeuroD2 and/or inhibiting miR-210 may potentially be a simple and efficient therapeutic strategy to inhibit the malignant phenotype of GBM cells.