

Abstract

Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in humans. Despite of advances in medical management of solid tumors, the mortality rates of GBM patients remain high, which urge for a better understanding of GBM pathogenesis and improvement in its therapeutic strategy. Hypoxia has been correlated with the aggressive form of glial tumors, their poor prognosis and resistance to various therapies. MicroRNAs (miRNAs) have emerged as key players in cellular transformation and tumorigenesis and have shown great potential for cancer diagnostics and therapeutics. The present study is based on previous studies from our lab showing alteration in the miRNA profile in GBM cell line U87MG in response to severe hypoxia. Here, we take forward our previous studies to identify hypoxia regulated miRNAs that play critical role in hypoxia signalling and studying its clinical implications. A total of sixteen miRNAs were further validated by qRT-PCR and nine were found to be hypoxia regulated in GBM cells. Among these, miR-196a was found to be highly induced in response to hypoxia in a HIF dependent manner. miR-196a was also found to be significantly up-regulated in TCGA-GBM and Indian GBM patient cohorts. The high expression of miR-196a was shown to be associated with poor prognosis in GBM patients. We did functional characterization of miR-196a in GBM cell lines, U87MG and A172 using both inhibition and overexpression approach in normoxia as well as hypoxia. miR-196a overexpression was found to induce cellular proliferation, migration and colony forming potential and inhibit apoptosis in U87MG and A172 cell lines while miR-196a inhibition using anti-miR-196a showed opposite results suggesting oncogenic role of miR-196a in GBM. We further identified targets of miR-196a using a combination of bioinformatic and biochemical approaches. Notably, we found that miR-196a not only downregulates tumor suppressor genes but may also be involved in up-regulating the levels of specific oncogenes. We further unveiled that NRAS, AJAP1, TAOK1 and COL24A1 are direct targets of miR-196a. Our study also reported complex competitive regulation of oncogenic NRAS by miR-196a, miR-146a and let-7g in GBM. Analysis of microarray gene expression data obtained by miR-196a inhibition under hypoxia elucidated the role of miR-196a in HIF, Calcium Adhesion, Wnt and Cell Adhesion pathways. Interestingly, miR-196a was found to positively regulate the expression of various genes involved in induction or stabilization of HIFs and maintenance of hypoxic conditions, thereby suggesting the existence of an indirect miR-196a/HIF positive loop under hypoxia. Overall, our work identifies novel axis of HIF-1/miR-196a/NRAS in GBM and suggests its prognostic and therapeutic significance.