

ABSTRACT

Glioblastoma (GBM) continues to be the most lethal cancer type of the central nervous system with patients exhibiting a median survival rate of a meagre 14 months post diagnosis. Currently practiced therapeutic regimen (surgical resection with chemo-radio therapy) has proven inadequate in extending the lifespan and/or quality of life of GBM patients. A detailed understanding of molecular alterations governing GBM is thus essential for development of novel therapeutic options. Recent evidence has highlighted the importance of deregulated activity of chromatin remodeler EZH2 and telomere maintenance mechanisms in GBM with both implicated in gliomagenesis. However, a detailed understanding of their regulators and mediators remain poorly explored in GBM. In this report, through analyses of the ChIP-seq data performed using H3K27me3 (EZH2) antibody in Indian glioma patient cohort we identified a novel microRNA target of EZH2 namely miR-490-3p in GBM. We showed that miR-490 was significantly downregulated in low and high grade Indian glioma patient samples, GBM cell lines, as well as in The Cancer Genome Atlas (TCGA) patient cohort. Its downregulation is mediated *via* EZH2-mediated histone methylation and upstream DNA methylation as confirmed from its upregulation post EZH2 siRNA and 5-Azacytidine treatment, respectively. Functional characterization revealed that miR-490 inhibited cell viability, tumorigenicity, migration and Epithelial-to-Mesenchymal-Transition (EMT) in GBM cells with downregulation of multiple EMT transcription factors and pro-migratory genes. We showed that miR-490 directly targeted TGFBR1 and TGIF2 of the TGF- β signaling. TGIF2, a novel target, was shown to promote migration and EMT that could partially be rescued by miR-490-3p overexpression. Besides, we also showed that miR-490 directly targeted TRF2, TNKS2 and SMG1, belonging to the telomere maintenance mechanism and increased telomere dysfunction induced foci formation suggesting that miR-490 regulates telomere maintenance in GBM. Overexpression of miR-490 also resulted in induction of global DNA damage as seen from 53BP1 foci formation and increase in p- γ H2AX levels. Also, miR-490 was shown to inhibit TRF2-mediated telomere maintenance hallmarks as seen by reduced stemness (SOX2 and SOX4 levels) and increased senescence (downregulation of SIRT1 and accumulation of H3K9me3 marks). It also initiated the downstream DNA Damage Response (DDR) leading to p53 pathway activation and induction of REST target genes TUBB3 and L1CAM. This response

was dependent on p53 status of cells. Interestingly, positive correlation of TRF2 with these hallmarks highlights the importance of miR-490 mediated targeting of telomere maintenance which could be of therapeutic importance in GBM.

Taken together, suppression of miR-490 is associated with events promoting glioma progression namely upregulation of EZH2 and telomere maintenance. The inhibition of TGF- β signaling and telomere maintenance by miR-490 in GBM assigns significance to miR-490 as a novel therapeutic agent in GBM especially since these pathways are cooperatively known to enhance GBM aggressiveness.