The Regulation of Proliferation and Programmed Cell Death in Patient GBM Stem-Like Cells by EGFR/GSK3b/PP2A Signaling

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Introduction

High grade gliomas are incurable brain tumors with a dismal prognosis. A small subpopulation of cells with stem-like characteristics located in a tightly controlled microenvironment, the perivascular niche, has been postulated to initiate and maintain the tumor's neoplasticity and contribute to its therapeutic resistance (1-4).The discovery of novel molecular targets contributing to the pathology of aggressive gliomas is urgently required. The study presented here aims to define the regulation of glycogen synthase kinase 3 beta (GSK3beta) activity in GBM biology by using stem-like cells derived from human GBM patients.

Methods

To evaluate the possible deregulation of GSK3b in glioblastoma, tissues from 68 GBM patients, 20 normal brains either neocortex or hippocampus and GBM stem-like cells were analyzed for GSK3b and EGFR/pI3kinase-mediated signaling mechanisms.

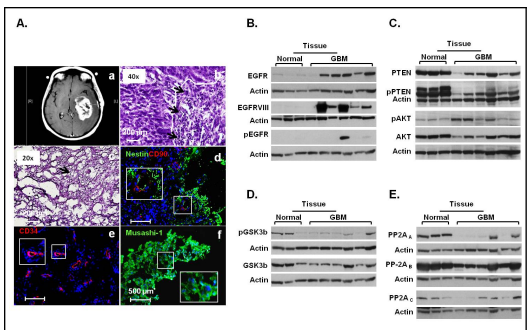

Figure 1. Deregulation of EGFR/pI3kinase/GSK3b signaling pathway in GBM patient tissue Representative protein blots show EGFR-mediated downstream molecules (B-E) while MRI (A-a), H&E (A-b/c) and Immunohistochemical examination (A-d-f) demonstrate characteristics of GBM and the expression of stem cell/progenitor/GBM markers.

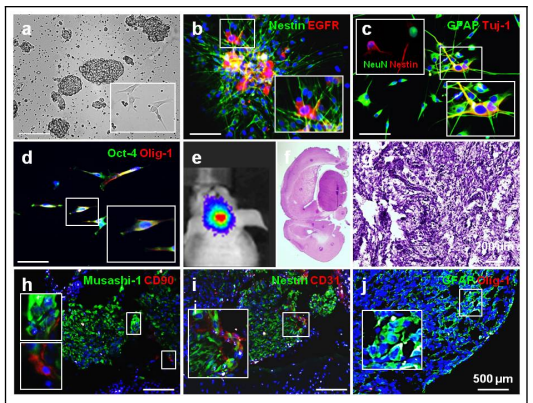

Figure 2. Phenotypic and molecular signatures of GBM stem-like cells. GBM patient-derived cells (a) show the features of stemness and became differentiated upon induction (b-c-d), highly tumorigenic (e-f), closely mimic human GBM (g) and retain the stem cell and GBM characteristics (h-i-j).

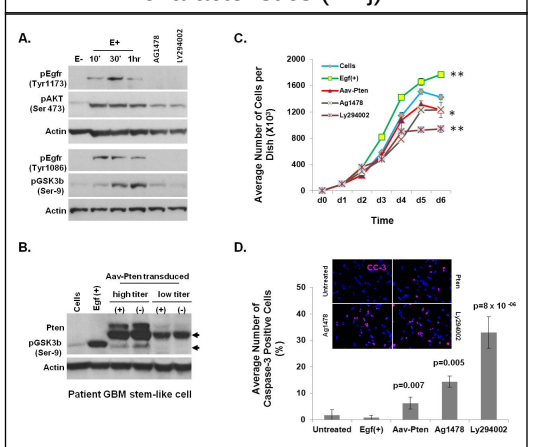

Figure 3. Activation of EGF receptors increases the phosphorylation of GSK3b at S-9 (A-B). Active GSK3b (dephosphorylated at S-9) attenuates GBM stem-like cell proliferation (C) and stimulates cell apoptosis via caspase-3 (D). P values are calculated with respect to untreated cells *=p<0.05, **=p<0.001 (C).

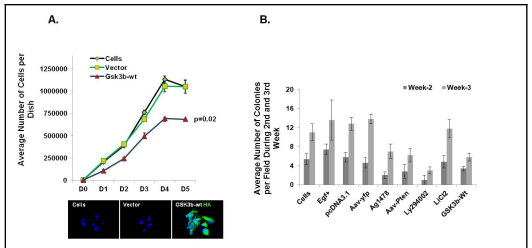

Figure 4. Cells overexpressing wild type GSK3b attenuates cell proliferation and inhibits colony formation (A-B). Immunostaining exhibits the expression and localization of GSK3b.

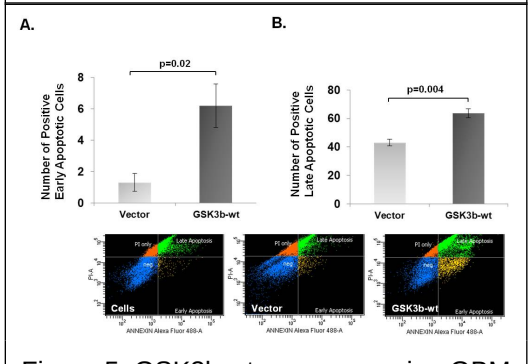

Figure 5. GSK3b wt overexpressing GBM stem-like cells exhibit stronger FITC Annexin positive staining than either untransfected or empty vector transfected cells. This data suggest that GSK3b wt overexpression in GBM stem-like cells stimulates early (A) and late (B) apoptosis.

Results

More than 60% of GBM patients downregulated GSK3b compared to normal tissue, which suggests a possible association of GSK3b deregulation in GBM pathology. GBM stem-like cells responded to exogenous EGF by phosphorylating EGFR, AKT and GSK3b. Blocking the phosphorylation of GSK3b at Serine 9 attenuated cell proliferation while stimulating apoptosis through cleaved Caspase-3.

Overexpression of GSK3b resulted in inhibition of cell proliferation, colony formation, and apoptosis, which strongly indicates an active role of GSK3b in tumorigenesis. To clarify the mechanism through which GSK3b may be regulated by cross-talk, GSK3b activity in GBM stem-like cells was knocked down and analyzed for the interaction of PP2A with GSK3b. Silencing PP2A on B subunit and/or inactivation of PP2A by okadaic acid interrupted GSK3b function, which suggests that PP2A may regulate GSK3b apoptotic activity.

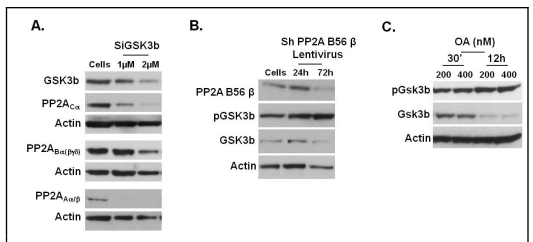

Figure 6. Silencing GSK3b results in down regulation of PP2A in patient-derived GBM stem-like cells (A). Knocking down PP2A on B56 subunit (B) or treating cells with OA (C) stimulates the phosphorylation of GSK3b.

Conclusions

Increasing evidence suggests aberrant signaling of EGFR, GSK3b and PP2A contributes to glial tumorigenesis. Therapeutic approaches targeting GSK3b in glioblastoma stem cells (GSCs) may assist in our treatment of GBM.

References

(1) Gürsel DB et al. Glioblastoma Stem-Like Cells-Biology and Therapeutic Implications. Cancers 2011 3(2):2655-2666.

(2) Pollard SM et al. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell 2009 4(6):568-80.

(3) Gürsel DB et al. Optimization of glioblastoma multiforme stem cell isolation, transfection, and transduction. J Neurooncol 2011 104(2):509-22.

(4) Hormigo A et al. A target for antiangiogenic therapy: Vascular endothelium derived from glioblastoma. PNAS 2011 108(11):4271-4272.

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