Epigenetic- and differentiation therapy display anti-cancer effects of EGFR/EGFRvIII-expressing brain cancer stem-like cells

Mikkel Staberg , Mette Villingshøj , Vladimir Berezin†, and Hans Skovgaard Poulsen

Introduction

- Glioblastoma Multiforme (GBM) tumors contain a subpopulation of cancer cells with stem cell properties termed brain cancer stem-like cells (bCSC)
- bCSC are involved in tumorigenesis, treatment resistance against conventional treatment and relapse
- The mutated and constitutively active version of EGFR, termed EGFRvIII, is proposed to serve as a bCSC marker
- Therapies targeting the EGFRvIII-expressing bCSC population could prove beneficial over standard treatments targeting the bulk tumor (Figure 1)

AIM

Investigate the ability of epigenetic- and differentiation therapy in targeting the EGFRvIII-expressing brain cancer stem-like cells

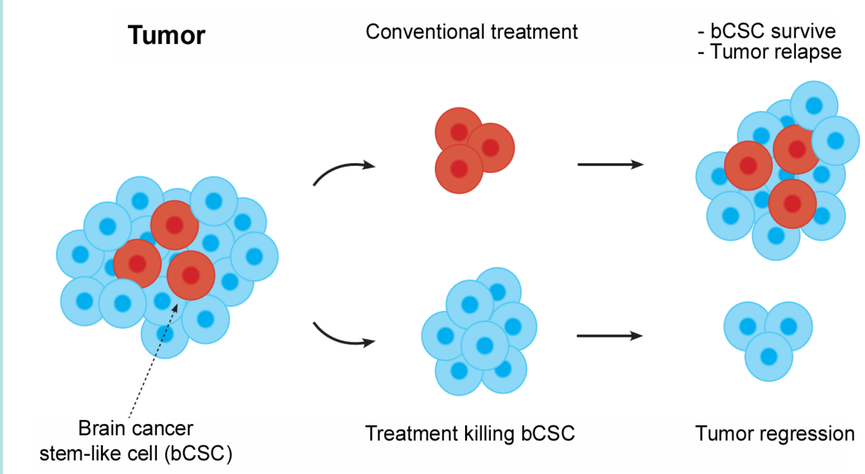

Figure 1:

Results

- Figure 2: Epigenetic treatment with TSA or AZA of GBM neurospheres influence expression of EGFR/EGFRvIII, Nestin and differentiation markers (CNPase, GFAP and βIII-tubulin) over time. (A) Representative WB showing protein expression. (B) Quantitative analysis of WBs normalized to house-keeping protein GAPDH and relative to control (1.0). (C) Q-RT-PCR showing mRNA levels.
- Figure 3: Differentiation-induced treatment with serum leads to decreased EGFR/EGFRvIII expression and upregulated differentation shown by WB (A) and Q-RT-PCR (B). TSA impairs serum-induced differentiation seen by a decrease in differentiation marker.
- Figure 4. TSA and AZA decrease clonal formation (A) and cell viability (B) in neurosphere cells in a dose-dependent manner. In addition, serum leads to decreased cell viability compared to control.

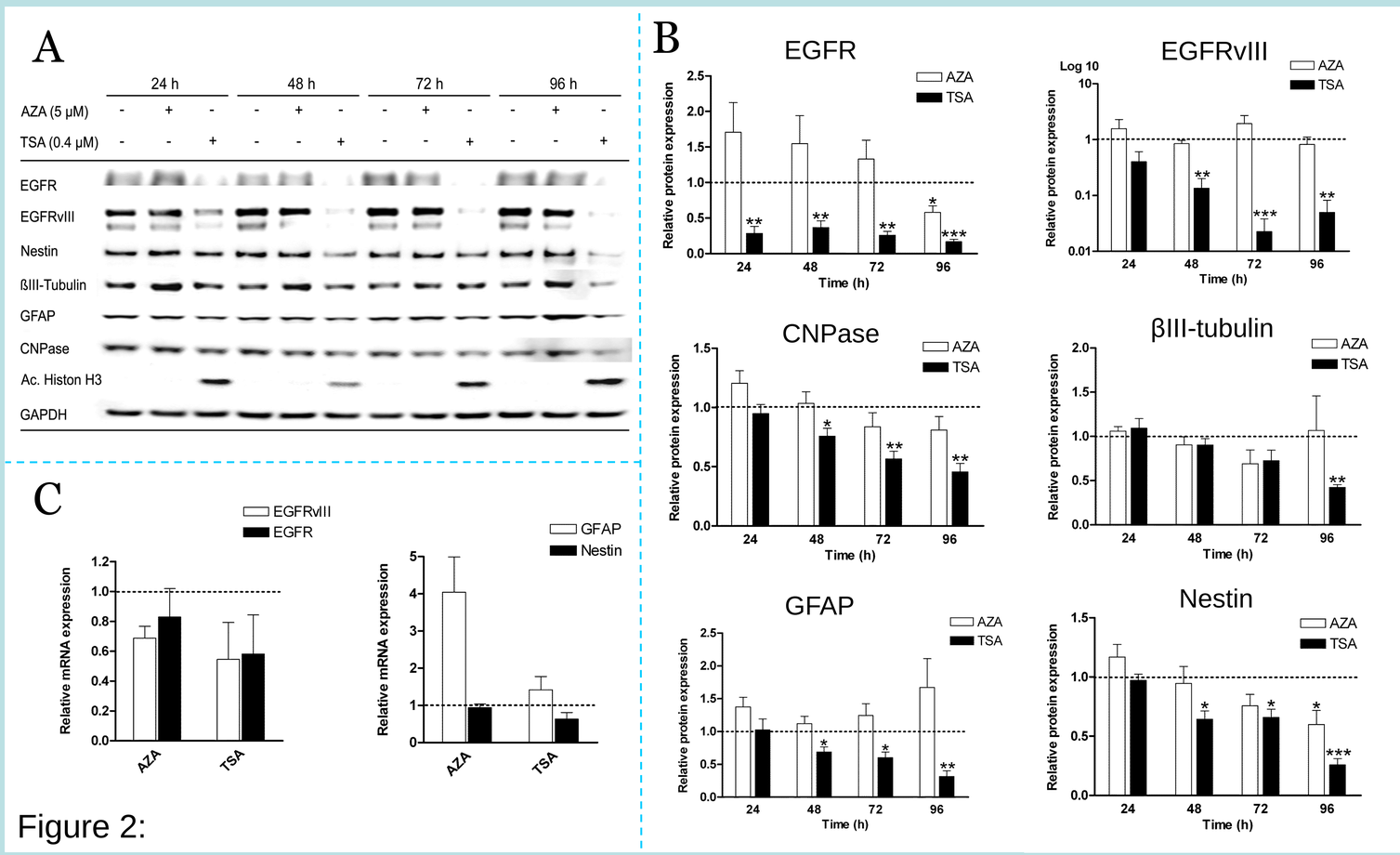

Figure 2:

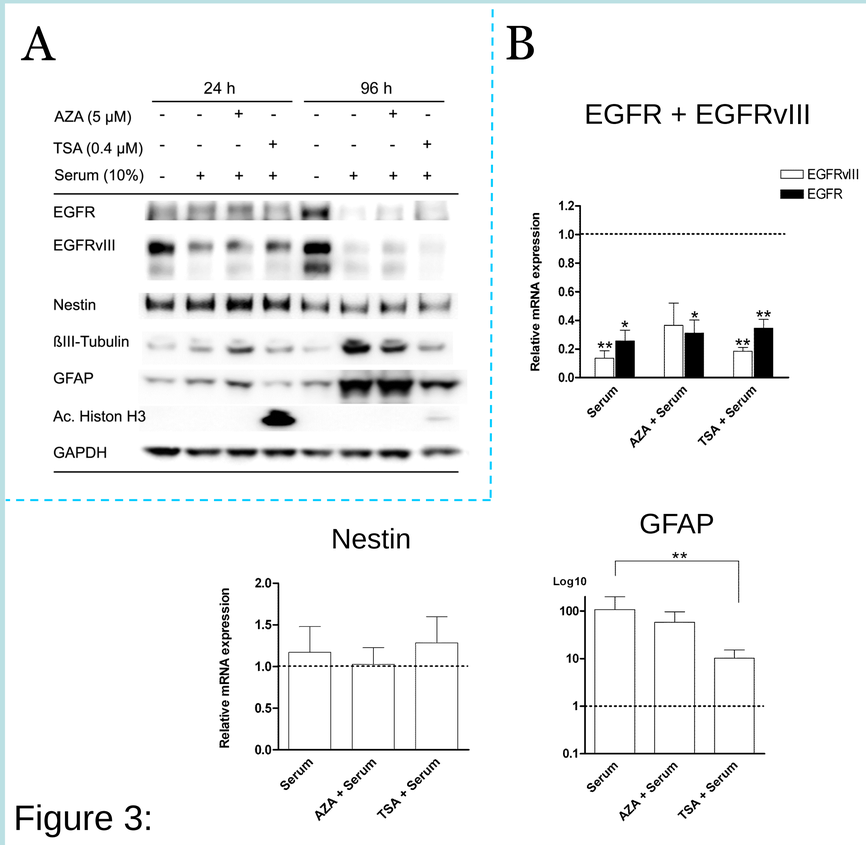

Figure 3:

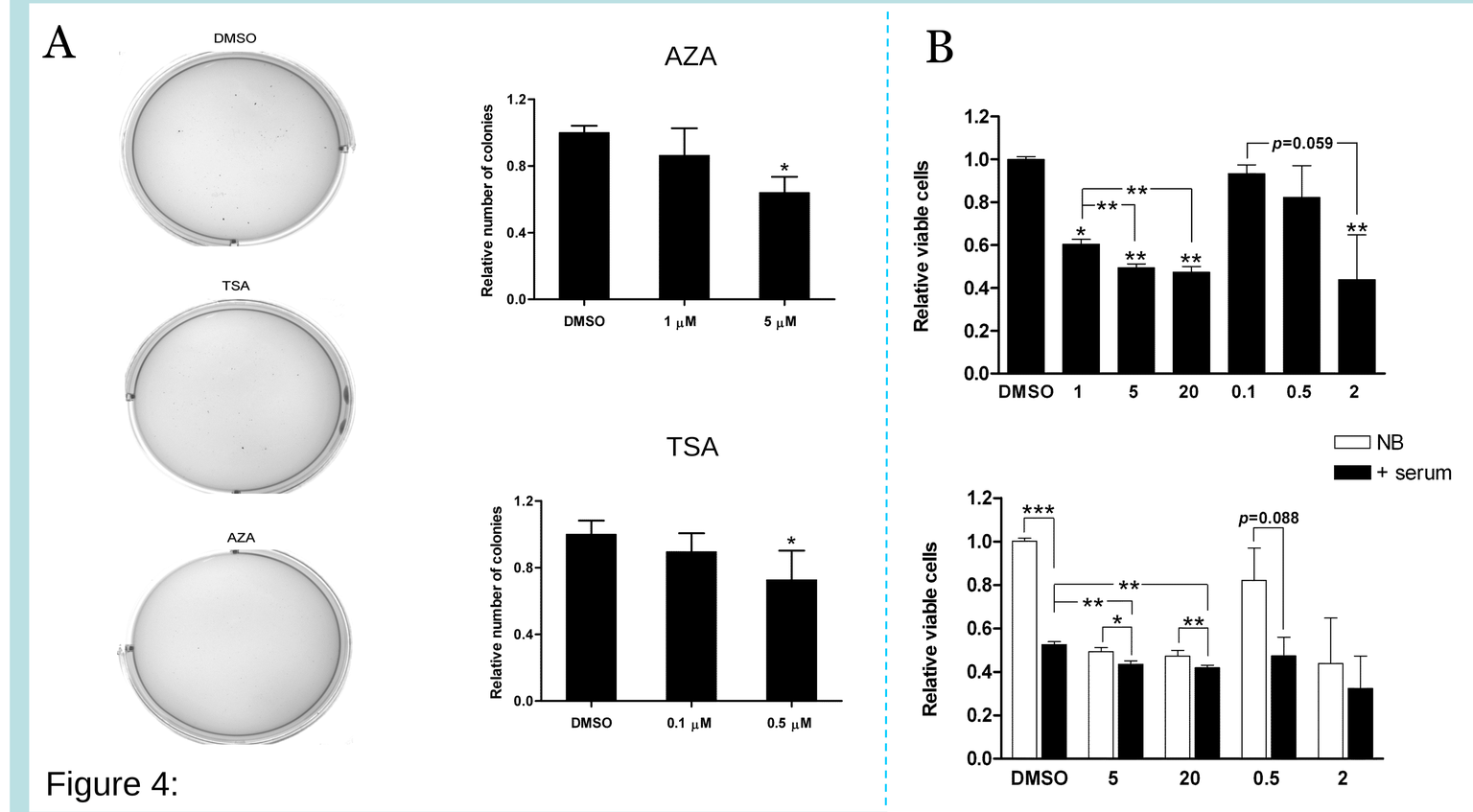

Figure 4:

Conclusions

- Both TSA, AZA and serum treatment decrease cell viability and influence the differentiation status of EGFRvIII-expressing brain cancer stem-like cells.
- Differentiation- and epigenetic therapy could prove beneficial over standard treatment by targeting the bCSC subpopulation thereby reducing relapse.
- These results provide a foundation for further investigation for use of differentiation and epigenetic treatments in GBM.

Methods

- The GBM cell culture used (CPH047p3m1) have been established from human derived primary GBM xenografts and was cultured as floating neuroshere cells in NB-media: Neurobasal™-A media supplemented with B-27, L-glutamine, EGF, bFGF, N2 and pen/strep. Differentiation of cells was induced by addition of 10% FCS in NB media.
- Epigenetic treatment was done by Trichostatin A (TSA) or 5-Aza-2-Deoxycytidine (AZA) dissolved in DMSO. Equal volumes of DMSO was used as a control.
- Protein expression was determined by Western blot analysis (WB).
- mRNA expression was analyzed by Quantitative Real-Time Polymerase Chain Reaction (Q-RT-PCR).
- Cell viability was measured by MTT assay.
- Tumorigenicity was estimated by clonal formation in a soft agar assay.
- Data showing significant difference are from at least three independent experiments.

,Department of Radiation Biology, The Finsen Center, Copenhagen University Hospital, Copenhagen, Denmark
† Department of Neuroscience and Pharmacology, University of Copenhagen, Panum Institute, Copenhagen, Denmark

email: mikkel.staberg@regionh.dk
www.radiationbiology.dk