CD97 Isoform Expression Distinguishes Glioblastoma from Lower Grade Gliomas and Confers An Invasive Phenotype

Michael Safaee BS; Aaron J. Clark MD PhD; Orin Bloch BS, MD; Michael E. Ivan MD; Taemin Oh BA; Matthew Z. Sun; Andrew T. Parsa MD PhD

Department of Neurological Surgery, Northwestern Universtiy, Department of Neurological Surgery, UCSF

Introduction

Tumor invasion limits surgical resection and confers worse survival in patients with glioblastoma (GBM). CD97 is an EGF-TM7 adhesion G-protein-coupled receptor known to confer an invasive phenotype and poor survival in patients with GBM. Transcript splicing of CD97 produces isoforms with variable EGF domains: EGF(1-5), EGF(1,2,3,5), and EGF(1,2,5). The later two possess similar ligand-binding properties and promote invasion and angiogenesis through integrin binding. The significance and expression pattern of these isoforms in glioma has not been described.

Methods

RNA and protein were isolated from U251 and U87MG cell lines, frozen tumor specimens, and GBM-derived brain tumor initiating cells (BTICs) isolated from patients treated at our institution. PCR was used to identify and sequence isoforms and perform quantitative analysis. Protein was assessed by Western blot and immunohistochemistry.

Results

U251 and U87MG cells express the EGF(1,2,3,5) and EGF(1,2,5) isoforms of CD97 as confirmed by sequencing (Figure 1A). Isoform splicing is schematically represented in Figure 1B. On Western blot, anaplastic and low grade astrocytomas express only small amounts of CD97 compared to GBM (Figure 2A). Transcript expression follows a similar pattern across grades with the EGF(1,2,5) and EGF(1,2,3,5) isoforms found in GBM (Figures 2B and 2C).

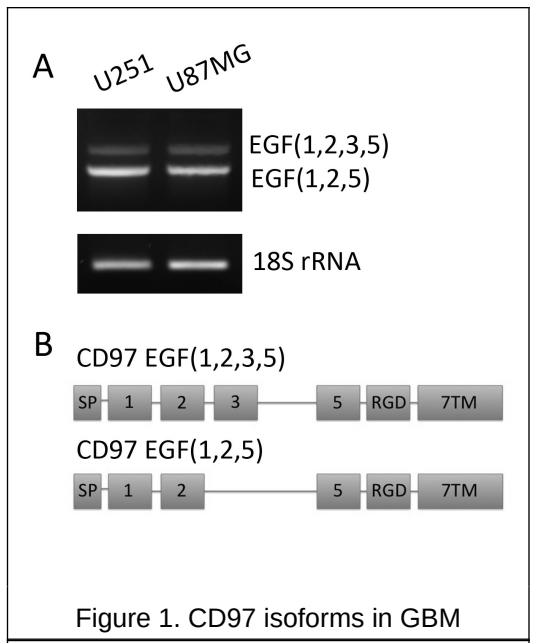

Figure 1. CD97 isoforms in GBM

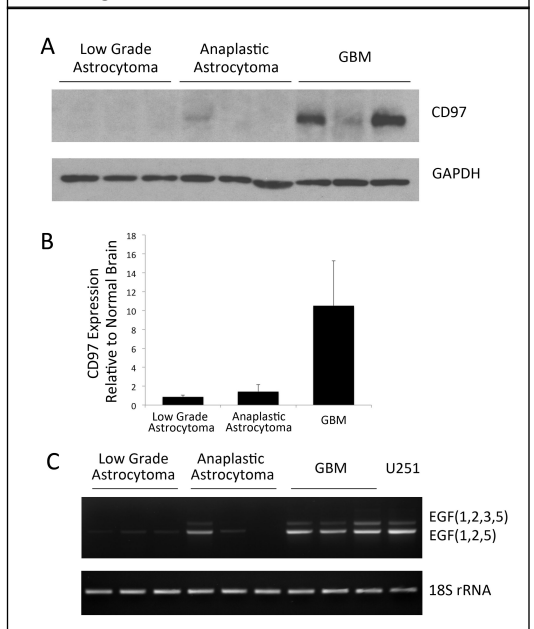

Figure 2. Expression of CD97 across histologic grade

Results

CD97 upregulation is highest among the classical (44%) and mesenchymal (25%) subtypes compared to neural (11%) and proneural (2%) subtypes ($p<0.001$, Figure 3). When stratifying by IDH1 mutation status, 62% of IDH1-mutant GBMs had downregulation of CD97 expression compared to 13% of IDH1 wild-type GBMs ($p<0.001$). Among GBM-derived brain tumor initiating cells, CD97 protein is expressed and localized to the cell surface (Figures 4A and 4C) with both isoforms present (Figure 4B).

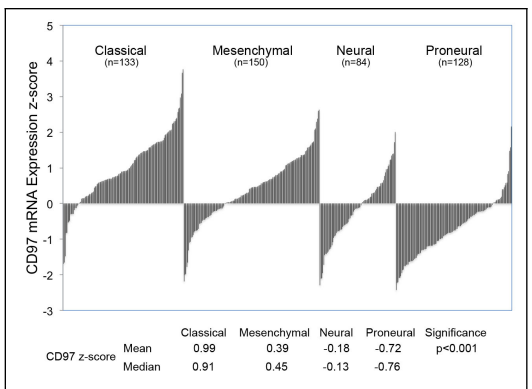

Figure 3. CD97 expression across genetic subtypes of GBM

Conclusions

CD97 confers an invasive phenotype and poor survival in GBM patients. Expression of the EGF(1,2,3,5) isoform is specific to GBM and absent in lower grade astrocytomas. CD97 expression is highest in the classical and mesenchymal subtypes of GBM and downregulated in tumors with IDH1 mutations. Future studies must focus on the viability CD97 as a therapeutic target.

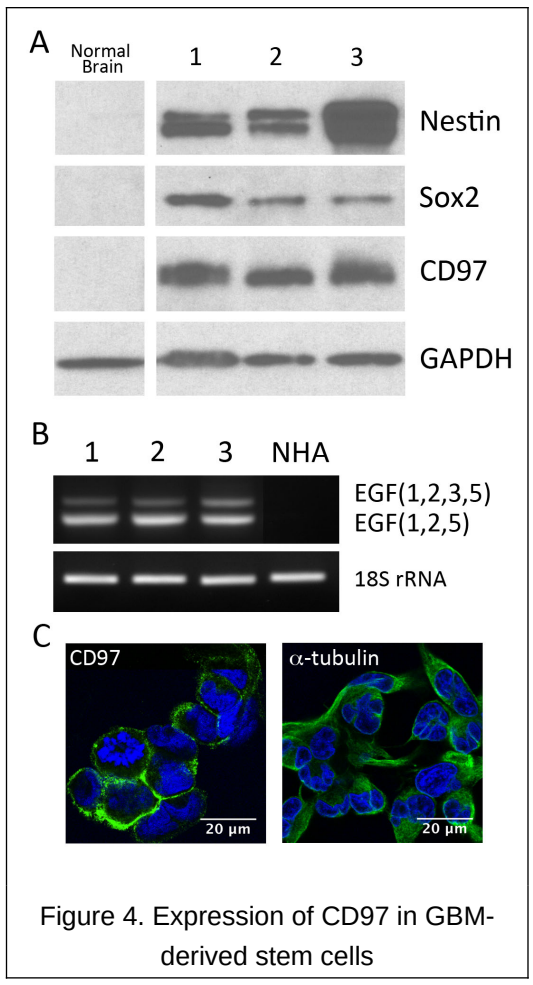

Figure 4. Expression of CD97 in GBM-derived stem cells

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of invasion in glioma, 2) Discuss the role of CD97 in glioma invasion, 3) Identify the importance of CD97 as a potential therapeutic target in GBM