Isolating Glioblastoma Stem Cell Pathways Through Phage Display Screening

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INTRODUCTION

The cancer stem cell hypothesis proposes that the ability of glioblastoma multiforme (GBM) to resist medical therapy lies in the cancer stem cell's (CSC) properties of self renewal, sustained proliferation, and tumor propagation.

Phage display technology is an unbiased target discovery platform that allows the isolation of a small subset of polypeptides that possess a specific binding affinity for a certain receptor or cell membrane to be selected from a larger library of random polypeptides. We have utilized phage display screening technology to isolate peptides that bind preferentially to glioma stem cells isolated from human GBM tissue. Analysis of these peptides has resulted in the discovery of novel proteins that serve vital roles in the glioma stem cell pathway.

METHODS

- in vitro & in vivo phage display screening
- protein BLAST peptide analysis
- rt-PCR gene expression analysis
- shRNA knockdown
- in vivo mice survival following intracranial xenograft injection

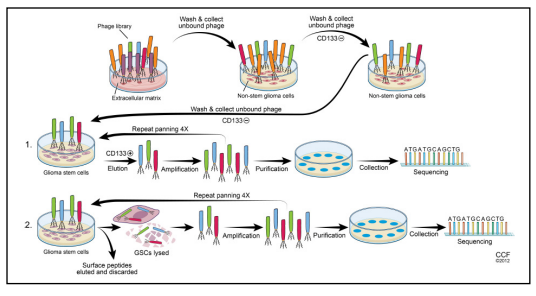

FIGURE 1. In vitro screening strategy

A phage peptide library was applied to an extracellular matrix coated plate. Unbound phage were collected and placed onto non-stem glioma cells for 'negative selection.' The resulting phage library was exposed to glioma stem cells cultured from human GBM tissue. In strategy (1), surface phage peptides were eluted with an acid elution. A total of 4 rounds of GSC binding was performed. Between each 'positive selection' round, the phage library was amplified to enrinch for the peptides with the strongest binding affinity. After 4 rounds, the phage peptides were collected and sequenced. In strategy (2), surface phage peptides were eluted and discarded. The remaining GSCs were lysed and the internalized phage peptides were collected.

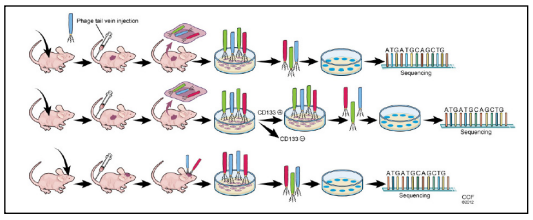

FIGURE 2. In vivo Screening Strategy

Glioma stem cells isolated from human GBM specimens were engrafted into SCID mice either subcutaneously into the flank or intracranially. After sufficient tumor growth, phage libraries are injected into the tail vein and allowed to circulate for 24 hours. Tumors were isolated and lysed to obtain tumor-bound phage peptides. In one experiment, tumor cells were dissociated to obtain the CD133+ cell fraction prior to lysing and collection of bound phage.

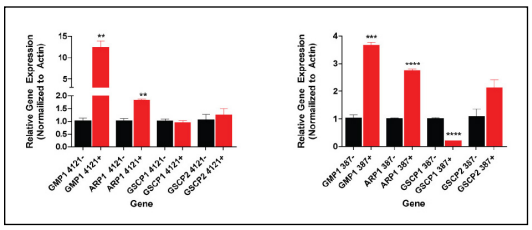

FIGURE 3. Non-stem vs Stem Cell Gene Expression

Peptide sequences were analyzed and matched with proteins using a BLAST search. 4 proteins were tested for expression in glioma non-stem versus stem cells. Glioma Motility Protein-1 (GMP1), Apoptosis Regulation Protein-1 (ARP1), Glioma Stem Cell Protein-1 and -2 (GSCP1 & GSCP2) expression were evaluated using rt-PCR in 2 different human GBM derived cell lines.

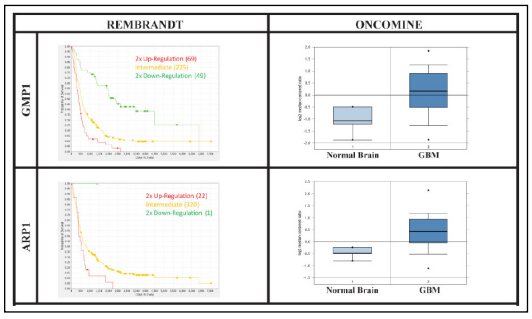

FIGURE 4. REMBRANDT Kaplan-Meier Survival and Oncomine Gene Expression Analysis

Glioma Motility Protein-1 (GMP1) and Apoptosis Regulation Protein-1 (ARP1) were entered into 2 patient databases to evaluate for their significance in GBMs: the REpository for Molecular BRAin Neoplasia DaTa (REMBRANDT) and Oncomine. REMBRANDT demonstrates Kaplan-Meier survival data based on gene expression and Oncomine compares gene expression between GBM and normal brain

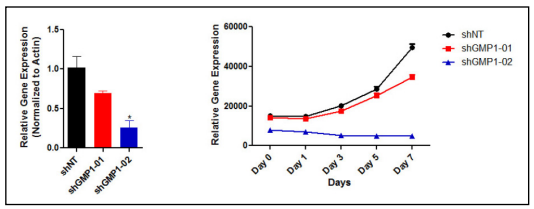

FIGURE 5. GMP1 knockdown and cell growth

GSCs transfected with GMP1 shRNAs showed decreased cell growth on cell titer assay.

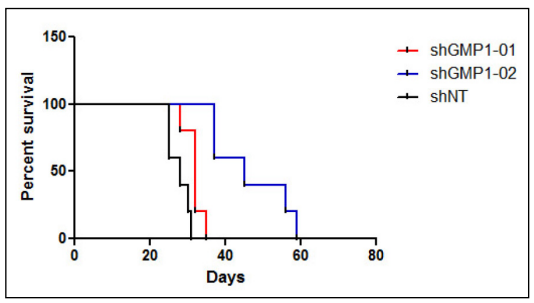

FIGURE 6. Survival data with *in vivo* intracranial knowckdown

GSCs transfected with GMP1 shRNA were intracranially injected into SCID mice. Kaplan-Meier survival curve demonstrates prolonged survial with knockdown of ARP1 and GMP1 expression

CONCLUSIONS

-Phage display screening can be effectively utilized *in vitro* and *in vivo* to isolate peptides specific for glioma stem cell binding

-Peptides obtained through screening analyzed via protein BLAST search can elucidate novel proteins involved in glioma stem cells specific pathways

-GMP1 are preferentially expressed in GSCs

-Knockdown of GMP1 in GSCs inhibit cell proliferation as well as prolong survival in *in vivo* mouse tumor models