Genetic analysis of CRISPR/cas9 basigin knockout T98 glioblastoma clones

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Abstract. The focus of the research in our laboratory is the transmembrane glycoprotein basigin. This interesting, multifunctional protein is involved in multiple cell processes, including embryonic development, embryo implantation into the uterus and metabolic regulation. Early studies demonstrated that basigin expression is elevated in rapidly growing cells such as cancer cells. This work suggested that releasing soluble basigin might stimulate the surrounding normal tissues to express a group of enzymes called extracellular matrix metalloproteinases (MMPs). MMPs play a central role in proliferation by breaking down the extracellular matrix surrounding layers of cells. We hypothesize that basigin expressed by cancer cells can induce MMP expression in normal tissues. To test this hypothesis, we sought to generate cell lines lacking a functional Basigin gene. Our lab used CRISPR/cas9 technology to attempt to disrupt the basigin gene (BSG) in T98 glioblastoma cells with the aim of eliminating basigin expression in these cells. For this, a Green Fluorescent Protein (GFP) DNA sequence was targeted for insertion into BSG. The work described herein shows the genetic analysis of the potential CRISPR/cas9 Basigin knockout clones using high-fidelity Polymerase Chain Reaction (PCR). Custom-designed oligonucleotide primers were used to amplify GFP DNA sequences from the T98 genomic DNA. The results of this study show that multiple independent T98 glioblastoma clones contain GFP DNA sequence in the genome. Future analysis of these clones will be performed to determine whether basigin protein expression was eliminated as a result of the CRISPR/cas9 targeting of the basigin gene.

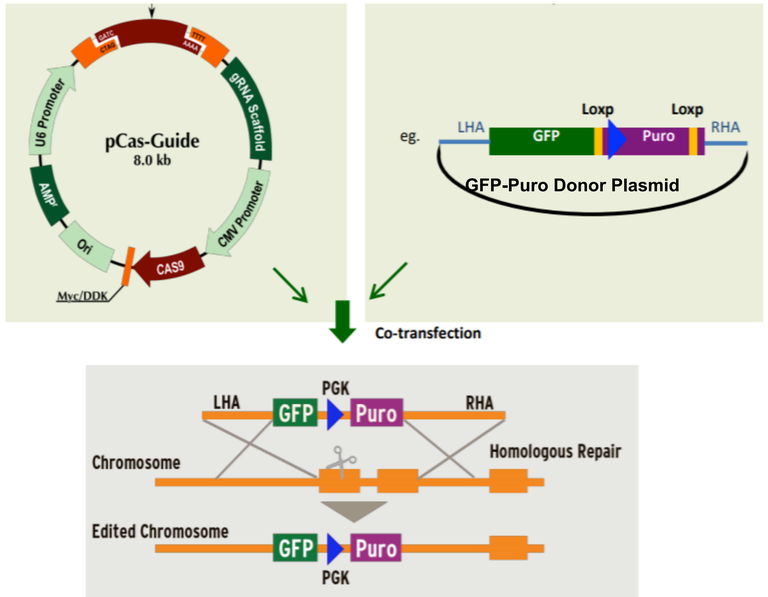

Figure 1. Knockout of the Basigin gene with the Origene CRISPR/cas9 'knock-in' approach. The pCas-Guide and the GFP Donor plasmids were cotransfected into cells. The pCas-Guide plasmid expresses the Cas9 enzyme which generates double-stranded DNA cuts to the Basigin gene. The GFP Donor plasmid possesses homologous Basigin DNA sequences (LHA and RHA) to promote insertion of the GFP DNA sequence through homologous recombination. GFP=green fluorescent protein; Puro=puromycin; PGK=phosphoglycerate kinase promoter; LHA=left homology arm; RHA=right homology arm.

Project Aim. Identify GBM clones where the Basigin gene is inactivated by the 'knock-in' of the GFP DNA sequence.

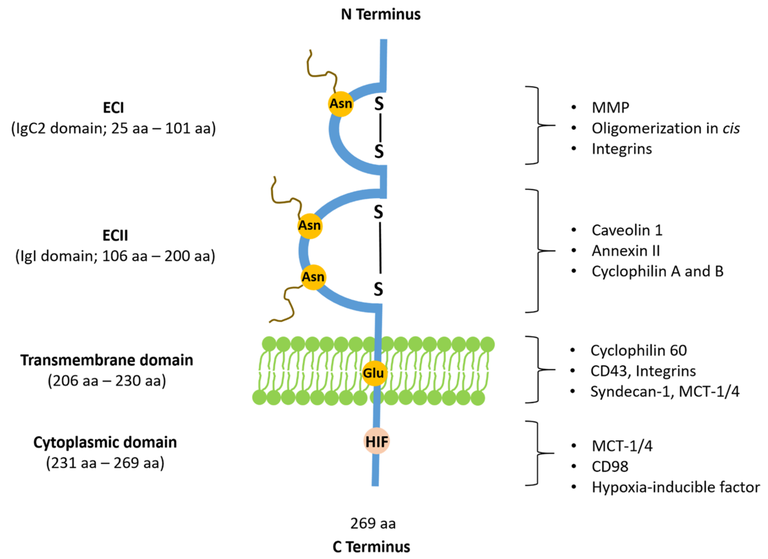

Figure 2. Basigin is a cell-surface transmembrane glycoprotein. The protein domains are listed on the left and the Basigin-interacting proteins on the right. The extracellular domain of Basigin possesses three asparagine amino acids that are heavily glycosylated. The extracellular domain is stabilized by disulfide bridges (S-S), and there is a charged amino acid (glutamic acid) present within the transmembrane domain of the protein. Adapted from Li & Nowak 2020.

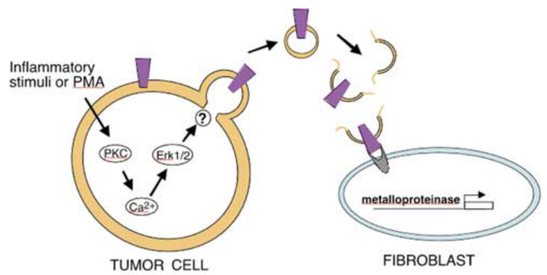

Figure 3. Basigin is is shed from the cell surface. The release of membrane microvesicles allows Basigin to diffuse away from the cancer cell where it can interact with the Basigin receptor on target cells. Adapted from Sidhu et. al 2004,

Experimental Methods. High performance polymerase chain reaction (PCR) using New England Biolabs Phusion polymerase uas used with custom-designed primers to identify the insertion of the GFP gene into cell chromosomes

Table 1: Designed oligonucleotide primer sequences used to perform PCR.

Primer	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
GFP	GGCTACGGCTTCTACCACTT	TTGAAGGCGTGCTGGTACTC

Experimental Results

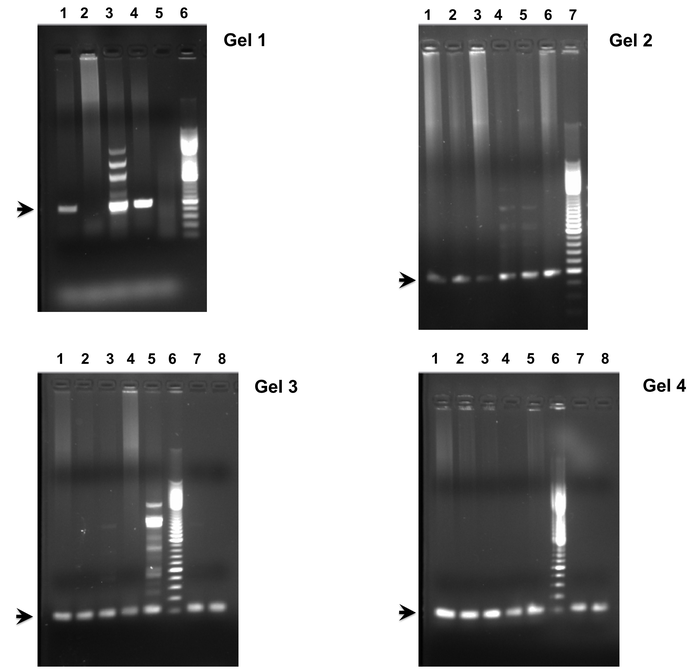

Figure 4. PCR analysis of genomic DNA from CRISPR/cas9 clones reveals that the GFP gene was inserted into the genome of T98 glioblastoma cells. The position of the expected band is shown with an arrowhead. Gel 1 shows the results of the PCR with clones 1-4 in lanes 1-4. Note that clone #2 in lane 2 does not have a GFP band. Gel 2 shows the results of the PCR with clones 5-10 where all of the clones possess a GFP band. Gel 3 shows the results of the PCR with clones 11-15 in lanes 1-5 and Gel 4 shows the results of the PCR with clones 16-20 in lanes 1-5. Note that the negative controls in lanes 7-8 gave positive results for GFP amplification. This suggests that the data from Gels 3 and 4 may not be reliable and must be repeated. A 100 base-pair ladder (Promega) DNA ladder was added to either lane 6 or 7 of the agarose gels to allow for the estimation of PCR product sizes

Conclusions. The CRISP/cas9 technique appears to have successfully integrated the GFP donor plasmid sequence into the genome of 19 out of the 20 clones isolated. Future work will confirm the integration of the GFP sequence into the Basigin gene. Future work will use these cells to isolate membrane microvesicles to assess the necessity for Basigin protein in stimulating MMP expression in fibroblasts.

References.

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