# Genome-wide CRISPR/cas9 Knock-out Screens in Human Glioblastoma Identify Genetic Vulnerabilities

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## Introduction

Glioblastoma is the most common primary brain tumor, with a poor prognosis. Identifying the genetic vulnerabilities of cancer is a novel method for discovering new cancer therapeutic targets. The genetic vulnerabilities of glioblastoma are poorly understood, however. The recent CRISPR/cas9 genetic knock-out approach has been proposed as a useful method for high-throughput screening of such genetic liabilities.

## Methods

We established human glioblastoma stem-cell lines from five patients with primary glioblastoma. The validity of these cell lines was confirmed by demonstrating expression of nestin and SOX2 with RT-PCR, and of CD133 with flow cytometry. Cell lines were subcutaneously injected into mice to track tumour growth in vivo. Lentiviral transfection of a cas9 vector followed by antibiotic selection led to generation of stable cas9-expressing cell lines. A genome-wide library containing 123, 411 CRISPR guide RNAs (six guide RNAs per gene, targeting 19, 050 genes) was lentivirally transfected into these cell lines. DNA was extracted from cells and sequenced with Illumina Hi-Seq at day 1 and day 25 after transfection.

## Results

These patient-derived stem cell lines displayed clonal sphere growth of between 42 and 61%, with a median time survival time of 40 days when transplanted into immunocompromised mice. High-throughput sequencing revealed significant depletion of CRISPR guide RNAs for 1190 genes, with a high degree of overlap between independent replicates for a given cell line. Analysis of gene function using ontology databases demonstrated that DNA repair pathways, RNA and protein synthesis, and regulators of cell proliferation were significantly depleted genes. Moreover, a number of genes specific for neuronal proliferation and differentiation were identified. Comparison with known genetic vulnerabilities of other cancer types revealed a specific profile of genetic liabilities unique to glioblastoma.

## Conclusions

We have identified a unique genetic vulnerability profile for glioblastoma using CRISPR/cas9, representing a panel of promising novel cancer drug targets.

### Learning Objectives

- Genetic screening with CRISPR/cas9 is an effective method for cancer gene discovery.

- Glioblastoma has unique genetic vulnerabilities which represent potential drug targets.