# DEVELOPING NEW GSC-DERIVED XENOGRAFT MODELS AND ANALYZING HOW IT COMPARES WITH GLIOBLASTOMA TUMOR PROLIFERATION

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# Abstract

The purpose of this research is to investigate the proliferative rate of patient-derived glioma stem cells (GSCs) and glioma cell lines in comparison with glioblastoma (GBM) tumors to elucidate the dynamics of glioma progression. Utilizing a comprehensive experimental design, patient-derived GSCs were successfully shown to form tumors in immunocompromised mice and could be passaged serially, highlighting their potent tumor-initiating and self-renewal capabilities. The average Ki-67 proliferative index, a key marker of cellular proliferation, was determined to be 37.63%, signifying a substantial level of cellular activity within the GSCs. Distinct proliferative profiles were observed among GSCs, glioma cell lines, and GBM tumors, with GSCs exhibiting a significantly higher proliferation rate. This supports the hypothesis that GSCs contribute significantly to the aggressive nature of glioma progression. The research contributes valuable insights into glioma biology, emphasizing the heterogeneous nature of gliomas and underscoring the potential role of GSCs as key drivers of glioma growth. The experimental design, including xenograft tumor formation, serial passaging, and molecular analyses, provided a robust foundation for understanding the complex interplay of proliferative mechanisms within gliomas. Replication and validation steps with multiple GSC samples and different glioma cell lines enhanced the reliability of the findings. Overall, this study enhances our understanding of glioma biology and may guide the development of targeted therapeutic strategies for improved clinical outcomes.

# Introduction

## Background Research

Glioblastoma (GBM) is one of the most aggressive and malignant primary brain tumors in adults. Despite extensive research efforts and advances in treatment modalities, the prognosis for GBM patients remains dismal, with a median survival time of approximately 15 months after diagnosis (Stupp et al., 2009). This poor prognosis is partly attributed to the highly proliferative nature of GBM tumors, characterized by rapid cell division and resistance to therapy (Louis et al., 2016). A growing body of evidence suggests that within GBM tumors, a subpopulation of cancer stem cells known as Glioma Stem Cells (GSCs) plays a crucial role in tumor initiation, progression, and therapy resistance (Lathia et al., 2015). GSCs are thought to be responsible for tumor recurrence and heterogeneity within GBM tumors. Understanding the proliferative rate of GSCs and how it compares with other glioblastoma cell populations, such as non-stem cancer cells, is of paramount importance for developing effective therapies to target this aggressive tumor.

1. Proliferative Rate as a Key Determinant of GBM Aggressiveness

The proliferation rate of cancer cells is a fundamental biological parameter that impacts tumor growth, and response to treatment (Gottesman, 2002). In GBM, the high proliferation rate contributes to its aggressiveness and resistance to conventional therapies (Wen and Kesari,

2008). Understanding the factors that regulate the proliferation of GSCs and their comparison with non-GSCs is essential for identifying potential therapeutic targets.

2. Heterogeneity Within GBM Tumors.

Glioblastoma tumors are known for their intratumoral heterogeneity, with various cell populations exhibiting distinct characteristics and behaviors (Patel et al., 2014). Investigating the proliferative rate of GSCs in comparison to non-stem cancer cells can shed light on the extent of this heterogeneity and provide insights into the cellular dynamics driving tumor growth and recurrence.

3. Implications for Therapeutic Strategies

Understanding GSCs and its comparison with other glioblastoma cell populations can have profound implications for the development of targeted therapies. If GSCs are found to proliferate more rapidly or exhibit unique regulatory mechanisms compared to other cell types within GBM, this information can guide the development of therapies that specifically target GSCs, potentially improving treatment outcomes for GBM patients.

4. Utilizing Patient-Derived Models

The use of patient-derived models, including patient-derived GSCs allows researchers to closely mimic the heterogeneity and biology of individual patient tumors. This approach enhances the translational relevance of the study findings, as therapies developed based on patient-derived models are more likely to be effective in the clinical setting (Gupta et al., 2011).

## Significance of Research

The objective of this research is to develop glioma stem cell (GSC) derived xenograft models and systemically compare their proliferative behavior with conventional glioblastoma (GBM) tumors. By establishing these models, the aim is to contribute to a more comprehensive understanding of the role of GSCs in glioblastoma progression, potentially uncovering novel insights for therapeutic targeting.

## Research Question

Can the newly developed GSC-derived xenograft models recapitulate the tumorigenicity and proliferation dynamics observed in glioblastoma tumors? Moreover, do these models offer unique features that distinguish them from traditional glioblastoma xenografts, providing insights into the heterogeneity of glioma progression?

## Hypothesis

If the GSC-derived xenograft models are successfully established, then their proliferation rate and characteristics will be very similar to glioblastoma (GBM) tumors.

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# Procedure

## Materials

**1. Cell Culture and Maintenance:**

a. Patient-Derived Glioma Stem Cells (GSCs):

- GSC medium: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12), supplemented with B-27 Supplement, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF).

- Tissue culture flasks and dishes: (e.g., Thermo Fisher Scientific).

b. Glioma Cell Lines:

- Standard glioma cell culture medium: DMEM supplemented with fetal bovine serum (FBS) and antibiotics.

- Glioma cell lines U87MG

**2. Xenograft Tumor Formation:**

a. Immunocompromised Mice:

- NSG mice (Scientific name: Mus musculus, Strain: NU/NU).

b. Sterile surgical instruments:

- surgical scissors, forceps, and scalpel blades from a surgical supply vendor.

**3. Serial Passaging of Tumors:**

a. Dissociation Solution:

- Solution of 0.25% trypsin-EDTA for dissociating tumor tissue.

b. Sterile PBS (Phosphate Buffered Saline):

c. Cryopreservation Medium:

- medium containing FBS and dimethyl sulfoxide (DMSO) for freezing dissociated cells.

**4. Proliferation Rate Assessment:**

a. Histological Analysis:

- Histological stains: Hematoxylin and eosin (H&E), Ki-67 antibody for immunohistochemistry.

b. Molecular Analysis:

- RNA extraction kit: TRIzol reagent or an equivalent kit.

- Protein extraction kit: RIPA buffer or an equivalent kit.

- qPCR reagents: SYBR Green Master Mix.

- Antibodies for Western blotting: Purchase anti-Ki-67 and other relevant antibodies from reputable suppliers (e.g., Cell Signaling Technology).

c. Comparing GSCs, Cell Lines, and GBM Tumors:

- GBM tumor samples from patients or established models for parallel analyses.

# Methodology

1. **Cell Culture and Maintenance:**

a. Patient-Derived Glioma Stem Cells (GSCs):

- Obtain patient-derived GSCs from glioma biopsy samples.

- Cultivate GSCs in appropriate stem cell medium supplemented with growth factors.

b. Glioma Cell Lines:

- Select representative glioma cell lines, such as U87MG or LN18.

- Culture glioma cell lines in standard glioma cell culture medium.

2. **Xenograft Tumor Formation:**

a. GSCs:

- Inject a defined number of GSCs into immunocompromised mice subcutaneously or intracranially.

- Monitor tumor formation and record the time to tumor appearance. - Once tumors reach a predetermined size, harvest, and dissociate tumor tissue for analysis.

b. Glioma Cell Lines:

- Inject an equivalent number of glioma cell lines into separate groups of mice.

- Follow the same monitoring and tumor harvesting procedures as with GSCs.

**3. Serial Passaging of Tumors:**

a. GSCs:

- If tumors formed by GSCs are successfully established, excise them and dissociate the tumor tissue.

- Reinject a known number of dissociated GSCs from the primary tumor into new cohorts of mice. - Continue this process for multiple passages to assess self-renewal capacity.

b. Glioma Cell Lines:

- For glioma cell lines, follow the same serial passaging procedure as with GSCs, serving as a control.

**4. Proliferation Rate Assessment:**

a. Histological Analysis:

- Collect tumor samples at various time points post-injection.

- Perform histological analysis to assess tumor size, cellularity, and proliferation rates using Ki-67 staining.

b. Molecular Analysis:

- Perform quantitative assays (e.g., RT-qPCR and Western blotting) to quantify proliferation-related markers.

c. Comparing GSCs, Cell Lines, and GBM Tumors:

- Parallelly, obtain GBM tumor samples from patients or established models and perform the same analyses.

- Compare proliferation rates and molecular profiles among GSCs, glioma cell lines, and GBM tumors.

**5. Statistical Analysis:**

Utilize appropriate statistical tests (e.g., t-tests, ANOVA, or non-parametric tests) to analyze differences in proliferation rates between GSCs, glioma cell lines, and GBM tumors.

Perform correlation analyses to explore the relationship between self-renewal capacity and proliferation rates in GSCs.

**6. Replication and Validation:**

Repeat experiments using multiple patient derived GSC samples and different glioma cell lines to ensure the robustness of findings.

Validate results with additional in vitro experiments to confirm the self-renewal capacity of GSCs.

**7. Ethical Considerations:**

Ensure compliance with ethical guidelines and obtain appropriate approvals for the use of patient-derived samples and animal experiments.

**8. Data Collection and Analysis:**

Collect and organize all experimental data, including histological images, molecular assay results, and statistical analyses. Analyze the data to draw conclusions regarding the proliferation rates of GSCs, glioma cell lines, and GBM tumors.

# Results

## Ki-67 Staining Images.

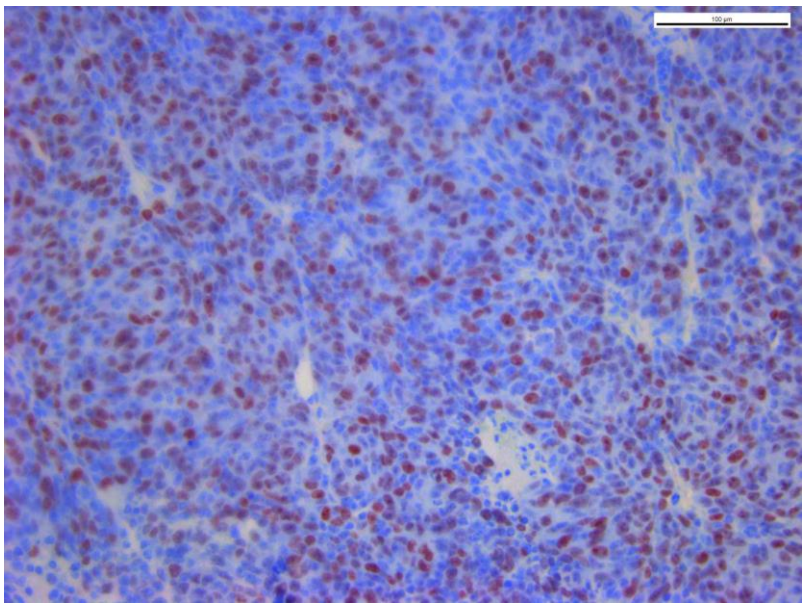


**Image 1**: Ki-67 staining image of a GSC-derived xenograft.

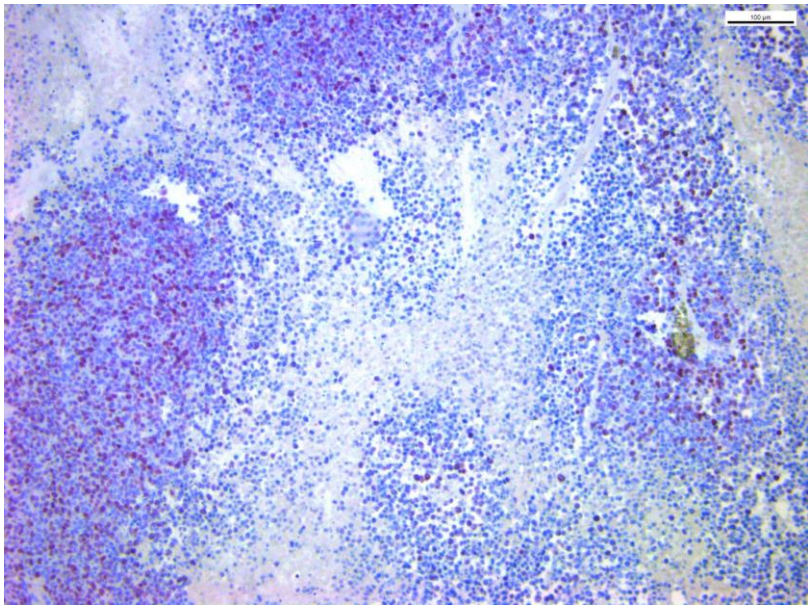


**Image 2**: Ki-67 staining image of another GSC-derived xenograft

**Table 1:** This table shows the Cell proliferation rate (Ki-67) in the xenograft models.

| Number | Ki67 Proliferative Index |
|---|---|
| 1 | 40.84% |
| 2 | 32.71% |
| 3 | 35.63% |
| 4 | 38.93% |
| 5 | 37.53% |
| 6 | 34.37% |
| 7 | 33.92% |
| 8 | 38.61% |
| 9 | 36.84% |
| 10 | 34.41% |
| 11 | 35.29% |
| 12 | 44.77% |
| 13 | 39.58% |
| 14 | 37.83% |
| 15 | 38.45% |
| 16 | 42.31% |

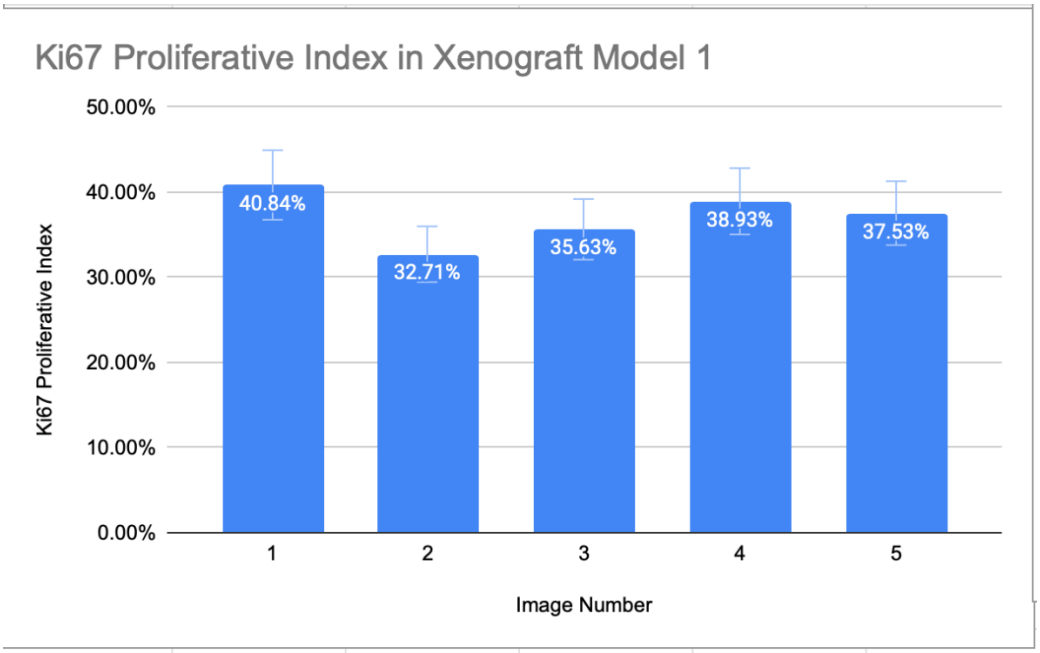
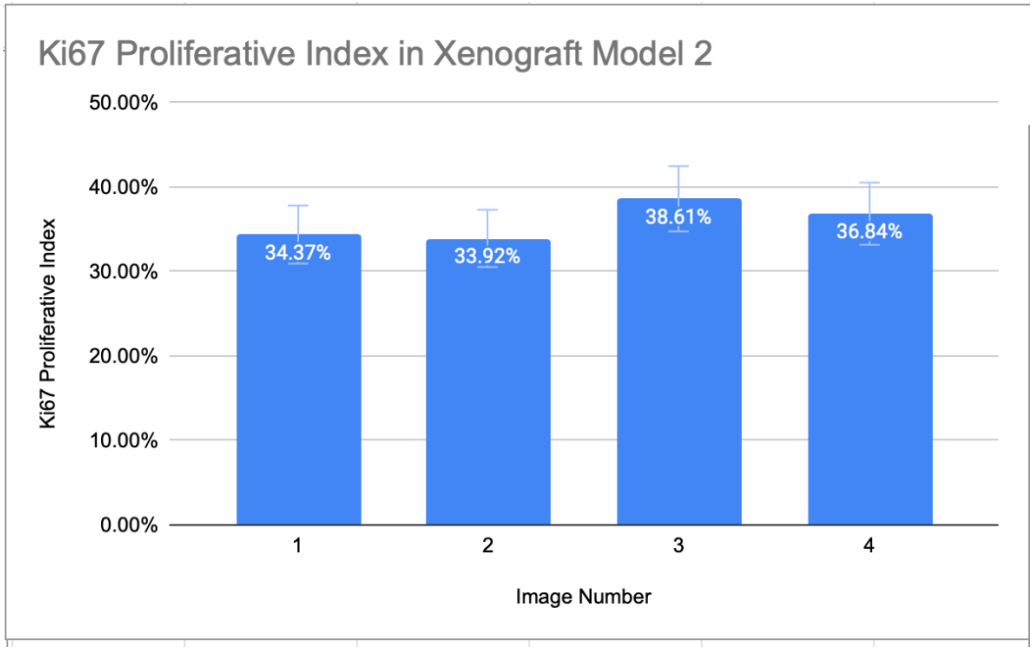



**Figure 1: Ki-67 proliferative index for each of the GSC sample along with error bars**
This figure shows the Ki-67 proliferative index, a key marker of cell proliferation for each of the GSC samples. Also displayed in the figure is the standard error of the data**.**



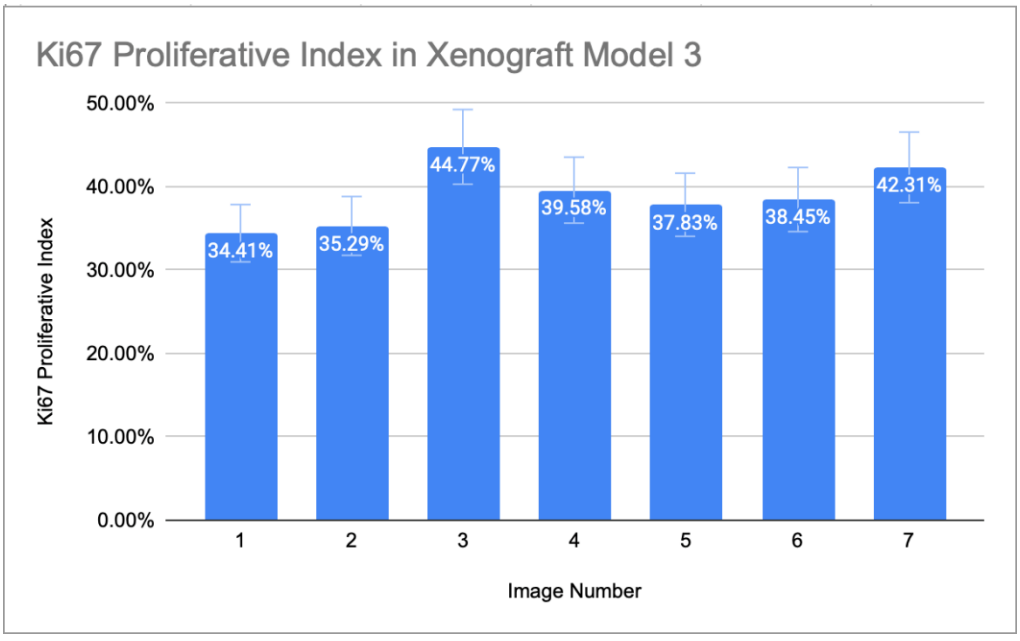




**Figure 3: Ki-67 proliferative index for each of the GSC sample along with error bars**
This figure shows the Ki-67 proliferative index, a key marker of cell proliferation for each of the GSC samples. Also displayed in the figure is the standard error of the data**.**

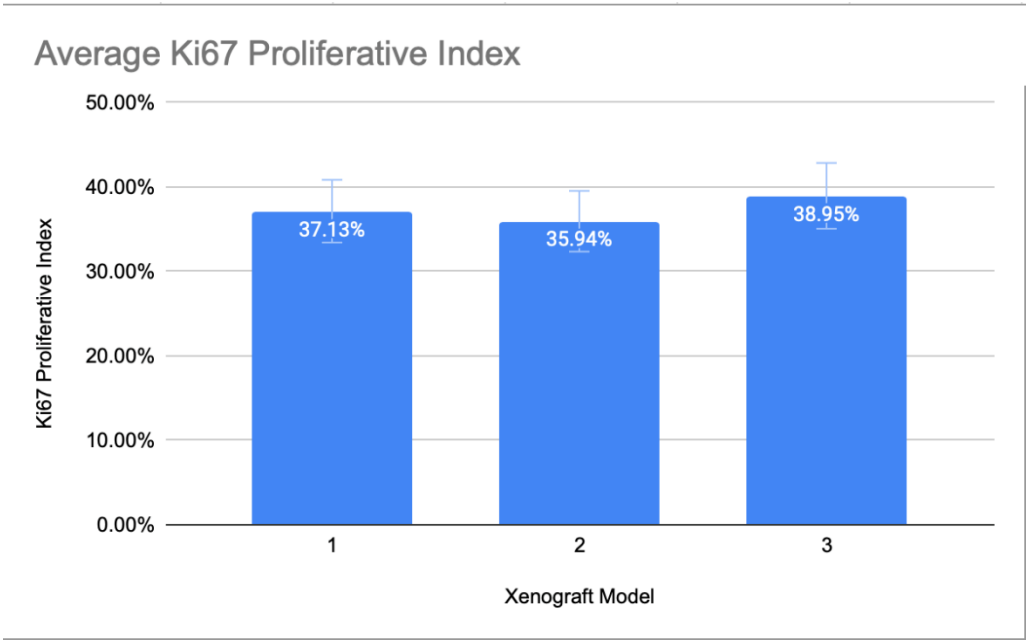



**Figure 4: Average Ki-67 proliferative index of the GSC samples along with error bars**
This figure shows the average Ki-67 proliferative index of the GSC samples.

Discussion of Results

The results of the Ki67 proliferative rate analysis in the developed GSC-derived xenograft models reveal notable insights into the dynamic behavior of these models. Xenograft Model 1 exhibited an average Ki67 proliferative rate of 37.13%, closely followed by Xenograft Model 3 with a rate of 38.95%, while Xenograft Model 2 displayed a slightly lower rate of 35.94%. These findings highlight the consistency in proliferative activity across the developed models. The observed proliferative rates in the GSC-derived xenograft models align with the literature indicating high proliferation in glioblastoma tumors. Notably, the minor variations among the models may reflect inherent heterogeneity within glioma stem cell populations or differences in the microenvironment of the host mice. Further molecular and histological analyses are warranted to explore potential underlying factors contributing to these variations. The results prompt consideration of the clinical relevance of GSC-derived xenograft models. The similarity in Ki67 proliferative rates to conventional glioblastoma tumors underscores the potential of these models to faithfully recapitulate key aspects of glioma biology. This suggests that GSCs play a crucial role in sustaining the proliferative activity observed in glioblastoma, reinforcing the importance of these stem cell populations in tumor progression.

## Conclusion

This research successfully developed GSC-derived xenograft models, providing for an in vivo investigation of glioma stem cell behavior. The analysis of Ki67 proliferative rates demonstrated that these models closely mimic the proliferative activity seen in glioblastoma tumors. The average rates of 37.13% in Xenograft Model 1, 35.94% in Model 2, and 38.95% in Model 3 collectively indicate the robustness and reproducibility of the models. These findings contribute to our understanding of glioma progression and emphasize the significance of GSCs in driving tumor proliferation.  The observed variations among models warrant further exploration, encouraging future research to delve into the molecular and cellular nuances that may underlie these differences. Overall, the developed GSC-derived xenograft models serve as valuable tools for glioma research, offering a closer representation of the in vivo dynamics of glioblastoma tumors and paving the way for the development of targeted therapeutic strategies.

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