The Role of the CXCL16-CXCR6 Pathway in the Migration of Human-Induced Spheroidal Neural Stem Cells *in Vitro*

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Introduction

- **Glioblastoma (GBM)** is an aggressive and incurable cancer of the central nervous system[1].

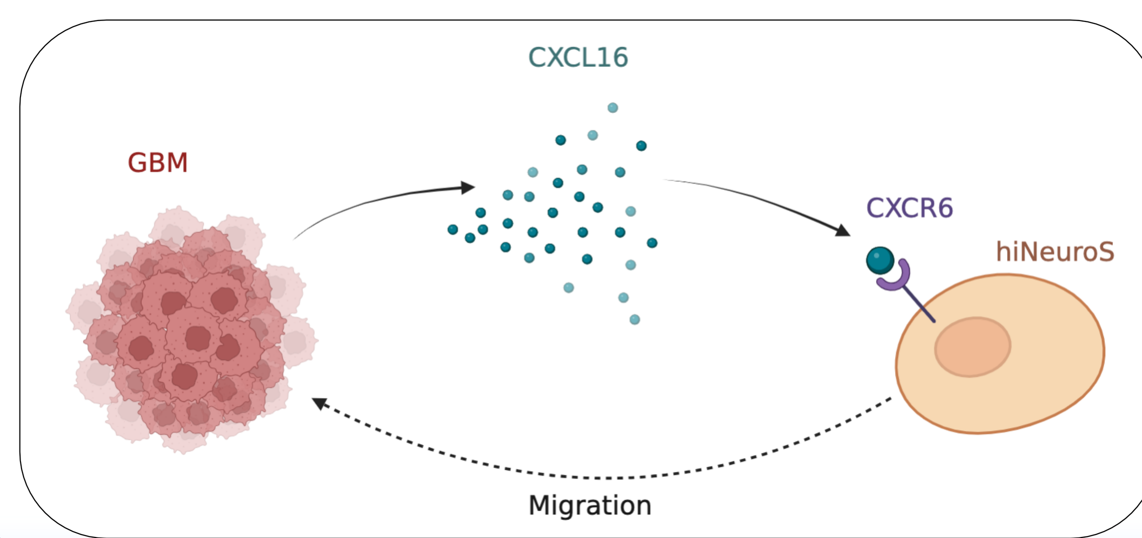
Median survival time: 15-23 months[2]	Tumor recurrence rate: 90% of patients[3]

- There are **numerous obstacles in treating GBM** that have impeded the progress of developing effective therapies:
  - x Rapid and extensive infiltration of surrounding brain tissue[4]
  - x Development of treatment resistance[5]
  - x Heterogenous tumor[4] + microenvironment[5]
  - x Immunosuppressive microenvironment[4]
  - x Upregulation of efflux pumps[4]
- **Human-induced spheroidal neural stem cells (hiNeuroSs)** are a promising cell-based therapy for GBM due to their robust innate tumor-homing characteristics[6].
  - ✓ Can be engineered to continuously secrete biotherapeutics
  - ✓ Allows for precise and persistent delivery of anti-tumor therapies to distant GBM tumor foci within 72 hours → reduces likelihood of tumor recurrence

Research Aim: Determine the mechanism behind hiNeuroS tumor-homing migration.

Hypothesis: The CXCL16-CXCR6 chemokine pathway plays a role in the tumor-homing capabilities of hiNeuroSs.

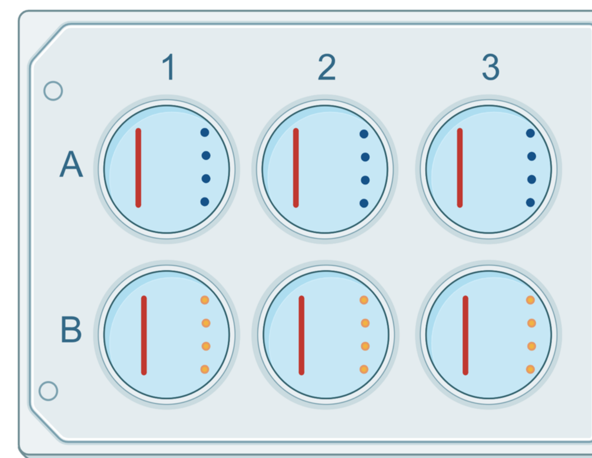
CXCL16:	CXCR6:
▪ Chemoattractant ▪ Overexpressed in GBM[7, 8]	▪ Receptor implicated in migration of GPCs[9] and MSCs[10]



Methods

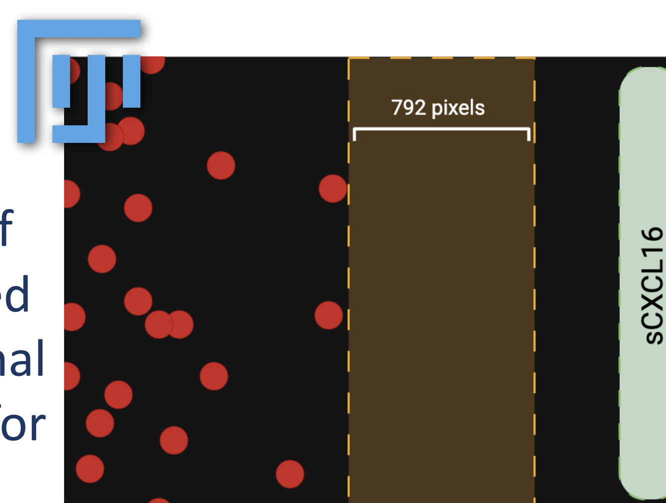
Agarose Gel Migration Assay:

1) 6-well plates prepared with 5mL agarose gel w/ ReNcell media per well. HiNeuroS-mCherry-FLuc seeded in channels on left side. On right side, 4 injections of either PBS or sCXCL16.



2) Fluorescent imaging using an EVOS M7000 imaging system @ 0, 6, 24, and 48-hours after treatment. 3 plates imaged with 3 images per well, per timepoint.

3) ImageJ software used to quantify hiNeuroS-mCherry signal in the region of interest, which started at the right-most signal in the 0-hour image for each location in the plate.



4) Statistical analysis using Jamovi software: Repeated measures ANOVA with post-hoc Tukey.

Results

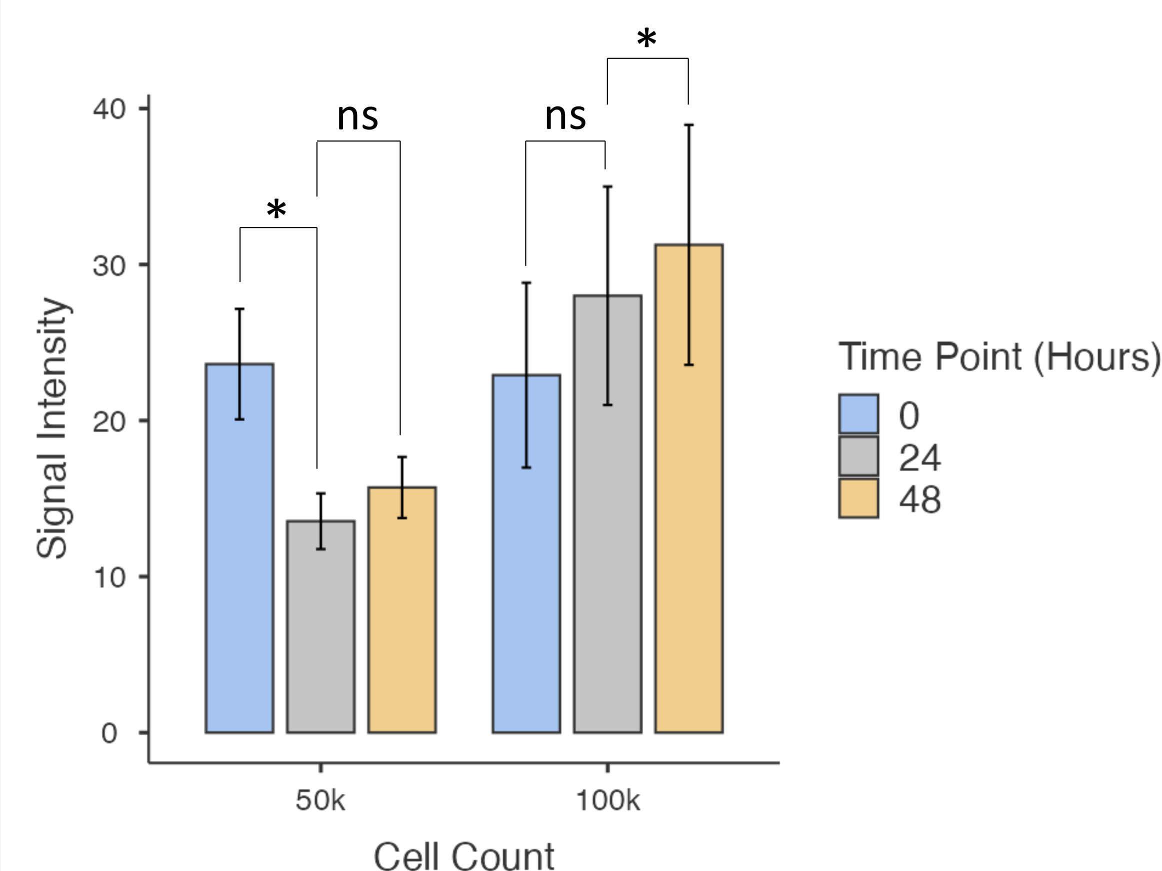


Figure 1. Viability Assay for hiNeuroSs in Agarose Gel. Fluorescent hiNeuroS-mCherry signal intensity measured at 0, 24, and 48 hours after seeding $5x10^4$ or $1x10^5$ cells in agarose gel channels in the absence of any treatment. N = 3 images were quantified for each treatment group at each timepoint. * = p < 0.05, ns (not significant) = p > 0.05.

Results Cont.

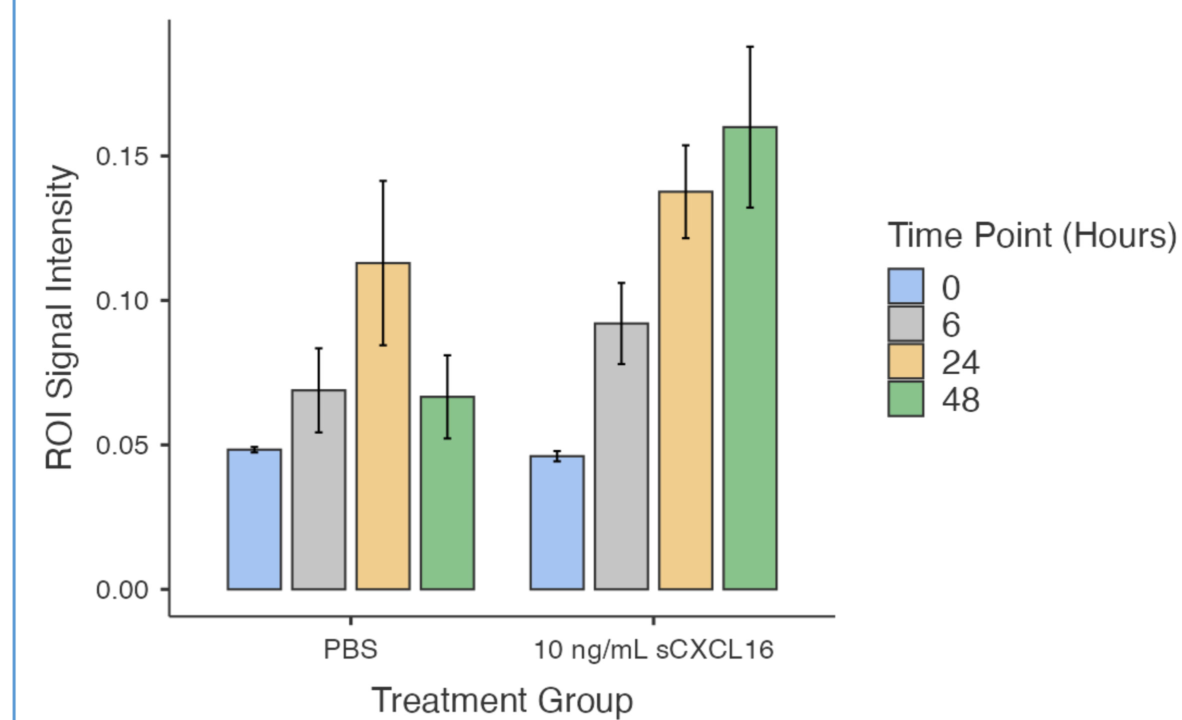
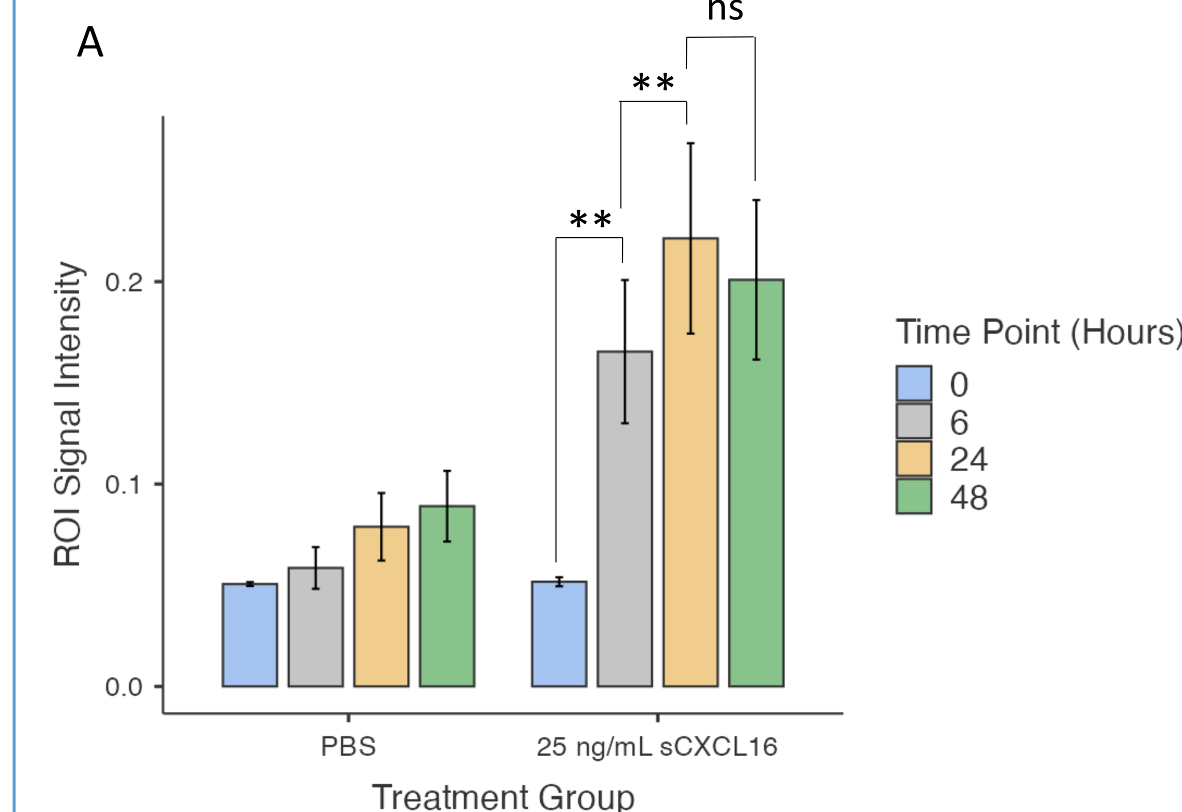


Figure 2. Agarose Gel Migration Assay with 10 ng/mL sCXCL16. Fluorescent hiNeuroS-mCherry signal within the ROI of each image measured at 0, 6, 24, and 48 hours for wells treated with PBS or 10 ng/mL sCXCL16. N = 216 images quantified.



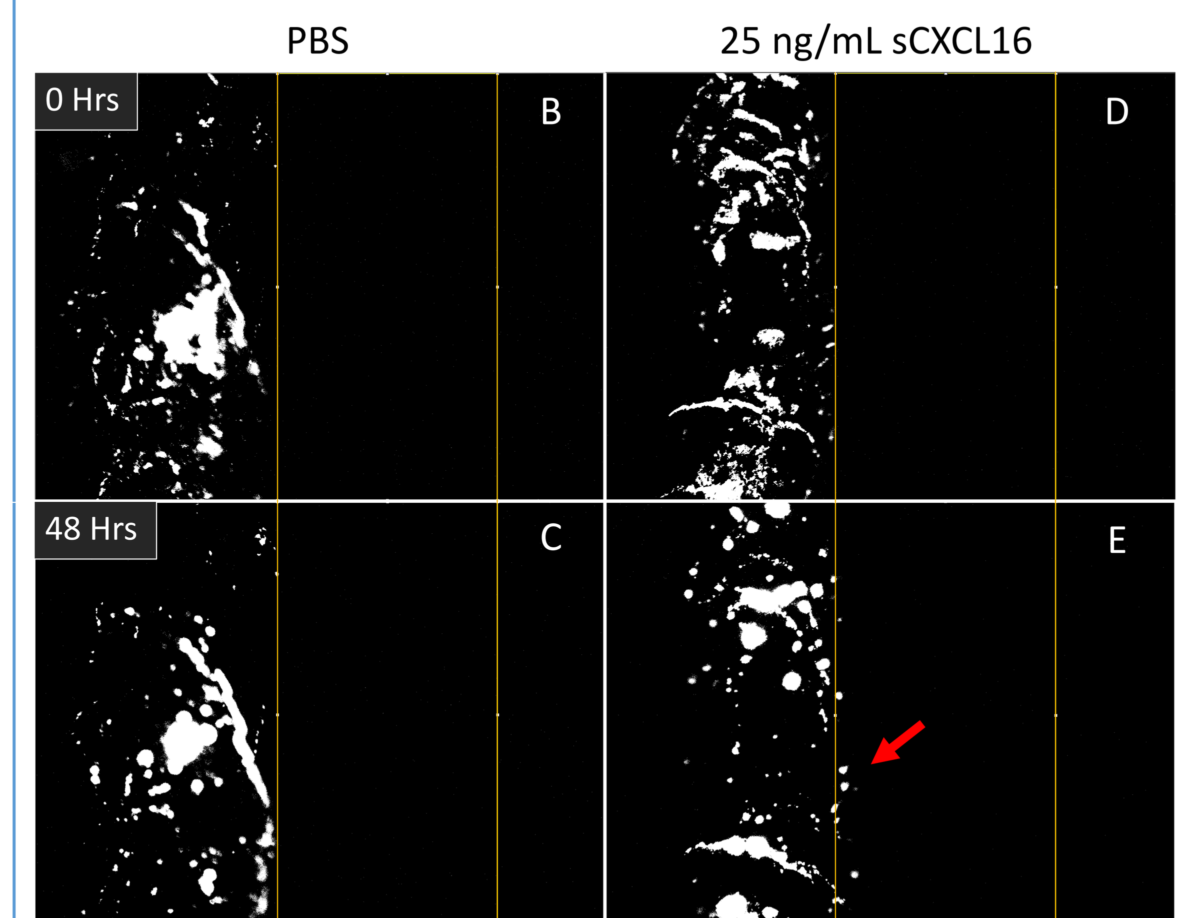


Figure 3. Agarose Gel Migration Assay with 25 ng/mL sCXCL16. A) Fluorescent hiNeuroS-mCherry signal within the ROI of each image measured at 0, 6, 24, and 48 hours for wells treated with PBS or 25 ng/mL sCXCL16. N = 216 images quantified. B-C) Representative images of hiNeuroS-mCherry signal at the same location at 0 and 48 hours with PBS, or D-E) 25 ng/mL sCXCL16 treatment on the right side. ROI represented by yellow lines. ** = p < 0.01, ns (not significant) = p > 0.05

Discussion

- ➢ **Novel agarose migration assay developed** to fit the unique characteristics of hiNeuroSs.
- ➢ **CXCL16 at higher doses (25 ng/mL) induced migration** of hiNeuroSs over 48 hours *in vitro.*

The CXCL16-CXCR6 axis may play a role in the tumor-homing capabilities of hiNeuroSs

- Implications:
  - Could inform further hiNeuroS optimization for the treatment of GBM
  - Novel agarose gel migration assay; may be applicable for the study of other migrating cell lines if the appropriate media is substituted
- Limitations:
  - Variability in shape of channels and distance from treatment
  - Possible cell death from lower cell count
- Future Directions:
  - Improve current study (increase cell count; increase time-course beyond 48 hours)
  - Antagonize CXCR6 in hiNeuroSs → Agarose migration assay
  - CXCR6 constitutive expression in hiNeuroSs → Agarose migration assay
  - CXCR6 constitutive expression in hiNeuroSs → *In vivo* migration studies

References

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