Engineered Microglia to Locate CD133+ Tumor-Initiating Cells

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Overall Goal

❖ Our goal is to engineer a microglia cell to seek and destroy the cancer-initiating cells in glioblastoma multiforme.

❖The engineered microglia will act as a modular machine with many future applications.

Abstract

Glioblastoma multiforme (GBM) is one of the most common forms of primary brain cancer which usually results in fatality. To date, it has been difficult to overcome primary brain cancer resulting from GBM, primarily because the cancer-initiating cells are suspected to be highly resistant to current cancer therapies. Specifically, CD133+ cells have shown resistance to hypoxia, irradiation, and some forms of chemotherapy. CD133+ hunting machines will be created by genetically engineering microglial cells (BV-2) with mammalian expression vectors. The project will also take advantage of inherent qualities of the microglia such as constant environmental sensing and quick motility. The engineered BV-2s will be equipped to locate the specific CD133+ GBMs and label the targeted cells with a tat-GFP fusion protein. It is the goal of this study to show an alternative approach to cancer treatment, and to emphasize the power of biologically available options to fight the disease.

Background

❖According to the WHO, glioblastoma multiforme (GBM) is a grade 4 tumor. Glioblastoma contain multiple types of cells, making them difficult to treat[2].

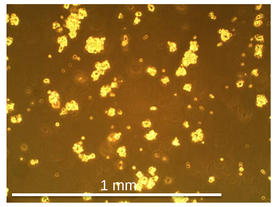
❖Current methods for tumor removal include: surgery, ultrasonic aspiration and polymer wafers, stereotactic radiosurgery, radiotherapy, and chemotherapy[2].

❖Microglia are brain macrophages located in the parenchymal space. They share many, if not all, of the properties of macrophages in other tissues, but they have a “ramified”, or branching out, morphology when in the resting state[3].

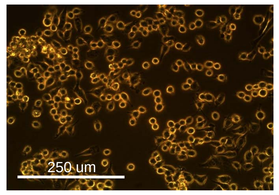
❖Cell-penetrating peptides (CPPs) are short peptide sequences that have the ability to penetrate the plasma membrane, allowing for the uptake of molecules. Molecules can range in size from nanoparticles to proteins, including even fragments of DNA. The first CPP discovered is known as trans-activating transcriptional activator (tat). It was discovered while studying HIV-1[1]. In this study, a fusion protein will be created by combining tat and green fluorescent protein (GFP) to act as a labeling mechanism of the CD133+ cells.

Materials & Methods

Cell Lines

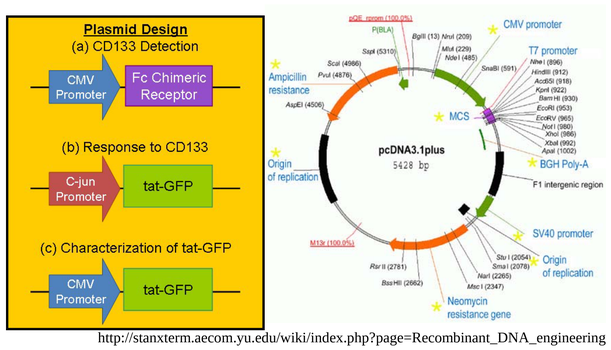


GAMB1: Primary Glioblastoma CD133+ cells. Kindly provided by Dr. P. Tofilon



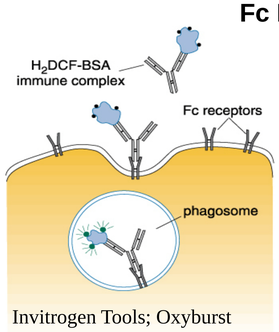
BV-2: Murine microglia cells. Kindly provided by Dr. D. Selkoe

Constructs



http://stanxterm.aecom.yu.edu/wiki/index.php?page=Recombinant_DNA_engineering

Fc Receptor



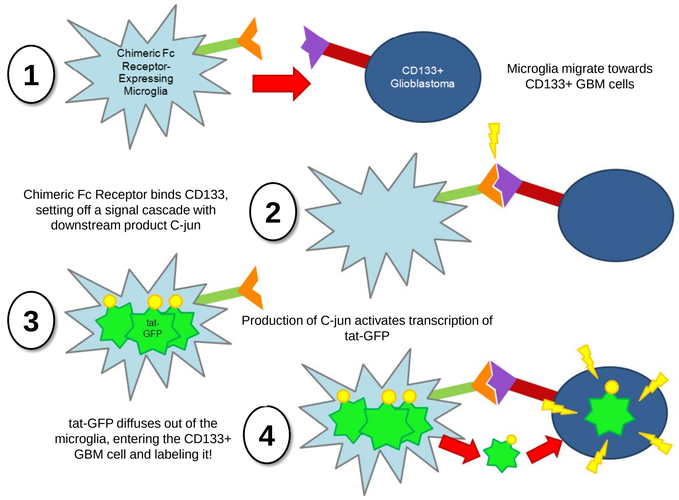
Invitrogen Tools; Oxyburst

To recognize the CD133+ cells we are engineering our microglia with a chimeric receptor using the Fc portion of the Fc γ receptor. Fc receptors recognize immunoglobulin and then activate a specific pathway, depending on the receptor.

Cell Culture

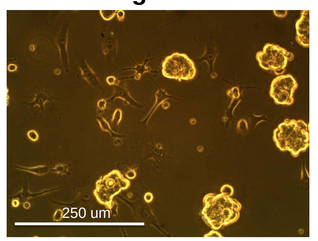
Cell Type	Reagent	Conditions
BV-2	RPMI 1640	37º C 5% CO2
	Gentamicin	
	10% Heat inactivated FBS	
GAMB1	D-MEM/F12	37º C 5% CO2 Poly-D-Lysine
	B-27 supplement	
	bFGF	
	EGF	

Experimental Design

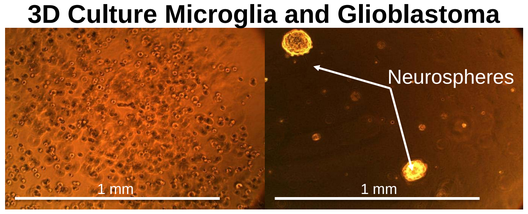


Data Collected

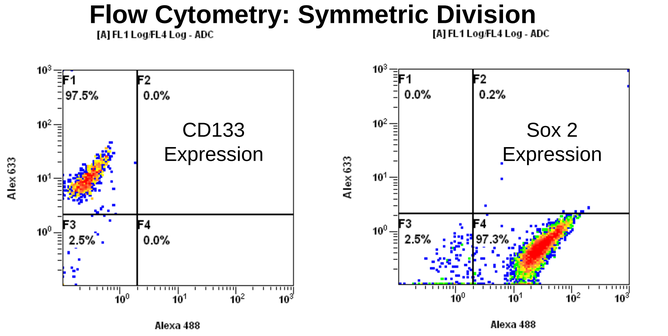
Co-Culture Microglia and Glioblastoma



3D Culture Microglia and Glioblastoma



Flow Cytometry: Symmetric Division



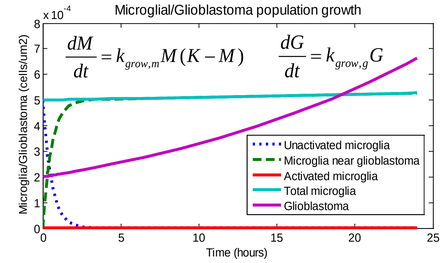
Region	Number	% Total	% Gated
ALL	1023	82.77	100
F1	997	80.66	97.46
F2	0	0	0
F3	26	2.1	2.54
F4	0	0	0

Region	Number	% Total	% Gated
ALL	5345	82.29	100
F1	0	0	0
F2	10	0.15	0.19
F3	136	2.09	2.54
F4	5199	80.05	97.27

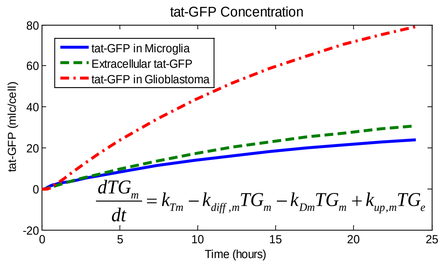
Modeling

❖**Purpose:** Predict and analyze system behavior

❖**Assumptions:** 2D system, Mass-action kinetics, No extracellular tat-GFP degradation



Variables:
- M = microglia
- G = glioblastoma
- TG = tat-GFP
  - m = microglial
  - e = extracellular



Parameters:
- k_{grow}= growth rate
  - m = microglia
  - g = glioblastoma
- k_{Tm} = production of tat-GFP per cell
- $k_{diff,m}$= diffusion of TG out of M
- k_{Dm}= degradation of TG in M
- $k_{up,m}$= re-uptake of TG by M

Future Work

The future work for this project involves a “seek and destroy” experiment in 2D culture. We want to create and introduce an Fc chimeric receptor to the microglia. We will then hook the two systems together to create a seek and label, and eventually a seek and destroy biological machine. The overall goal is a proof of concept for an alternative glioblastoma multiforme cancer therapy. This system is modular: it has the capability to be used for other problems by changing the target protein and receptor. The study will continue into 3D culture created from collagen.

References

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2. http://www.irsa.org/glioblastoma.html
3. R. Bryan Rock, Genya Gekker, Shuxian Hu, Wen S. Sheng, Maxim Cheeran, James R. Lokensgard, and Phillip K. Peterson, 2004 A.D. Role of Microglia in Central Nervous System Infections, *CMR***17**.4.(2004), pp. 942–964

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