Receptor targeted and encapsulated MSC for tumors in the brain

Khalid Shah

Massachusetts General Hospital
Harvard Medical School

Harvard Stem Cell Institute
Acknowledgements

Yanni Zhu
Dan Stuckey
Sung Hugh Choi
Wanlu Du
Clemens Reinshagen
Nusrat Jehan
Jordi Martinez
Deepak Bhere
Nabeel Nissar
Cody Cameron
Raj Vatsa
Jae Woo
Priyanka Ravikumar

Hiroaki Wakimoto, MGH
Jacob Hooker, MGH
Umar Mehmood, MGH

Ramon Alamay, Barcelona, Spain
Peter Sorger, HMS, Boston
Jack Lawler, BIDMC, Boston
Glenn Prestwich, Utah
Biotime
Human MSC migrate to tumors in the brain

MSC

Tumor

Week 2

Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy

Laura S. Sasportas, Randa Kasmieh, Hiroaki Wakimoto, Shawn Hingtgen, Gayatrty Mohapatra, Jose Luiz Figueroa, Robert L. Martuza, Ralph Weissleder, and Khalid Shah

Molecular Neurotherapy and Imaging Laboratory, Center for Molecular Imaging Research (CMIR), Department of Radiology, Departments of Neurosurgery, Neurology, and Pathology, and Center for Systems Biology, Department of Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

Sasportas and Kasmieh et al, PNAS 2009
Engineering Stem cells for tumor therapy

Secretion of therapeutic proteins

- Induce apoptosis
- Block proliferation
- Cell cycle arrest

Tumour cell

Direct

DR4 or DR5
EGFR
IFNR

TRAIL
EGF agonist
IFNα or IFNβ

Stem cell

Indirect

Immune effectors (IL-12, IL-18, IFNα and IFNβ)

NK cell
Macrophage
Fibroblast

Blood vessel effectors (PEX and aaTSP1)

T cell
Stromal effectors
Engineering Stem cells for tumor therapy

Stem Cell mediated suicide therapy

Stem Cell engineered to express CD, HSV-tk or CE

Cancer cells killed by bystander effect

Immune cells recruited by distant bystander effect

Stem Cell mediated nanoparticle delivery

Intracellular loading

Particle release: passive or active (photoinduction or hyperthermia)

Nanoparticle

Cell surface loading

Stuckey and Shah, Nature Reviews Cancer 2014
Receptor Targeted Therapies for tumors

TRAIL
Bimodal TRAIL/HSV-TK
EGFR Nb-TRAIL
Interleukin (IL)-13 – PE
IL24/MDA7
aaTSP1
Oncolytic herpes virus (oHSV)-TRAIL

Tumor cell
Endothelial cell
Tumor cell

Engineered Stem cell

S-TRAIL
Bi-specific ENb-TRAIL
IL13-PE
TSP-1
IL24/MDA7

Engineered Stem cell

Oncolytic herpes virus (oHSV)-TRAIL
Glioma cells (Fluc-mCherry)

Neural stem cells (TRAIL-IRES-GFP)

9’ intervals for 54h
Stem Cell released S-TRAIL induces apoptosis in glioma cells

Bagci et al, Oncogene 2012
Mouse model of glioma resection: Mimicking a clinical scenario of tumor debulking
Mouse model of glioma resection: Mimicking a clinical scenario of tumor debulking
sECM encapsulated SC migrate to tumors in the brain and induce apoptosis in tumor cells

Glioma cells

Encapsulated SC

Glioma cell viability

Caspase 3/7 activation

% glioma cell viability, Fluc intensity (normalized to control at 8 hr)

Fold changes in caspase-3/7 activity (normalized to control at 8 hr)

SC-GFP

SC-S-TRAIL

Control S-TRAIL

PARP

Cleaved Caspase 8 (full length)

Tubulin
sECM encapsulated SC- S-TRAIL significantly increase survival of mice with resected gliomas.
Bi-modal Stem cells co-expressing HSV-TK and S-TRAIL have anti-tumor effects and can be eradicated post treatment.

HSV-TK expression selectively sensitizes cells to ganciclovir (GCV) and can be utilized as a PET marker using [18F]FHBG as a substrate.
TRAIL sensitive and resistant GBM lines
Patient derived primary GBM cells have varying invading capabilities *in vitro* and *in vivo*.

**Primary lines**

- **GBM4**
- **BT774**
- **GBM8**

Day 1 | Day 3 | Day 5 | Day 8
--- | --- | --- | ---

**Established lines**

- **U87**
- **LN229**

Day 1 | Day 3 | Day 5 | Day 8
--- | --- | --- | ---

---

**Patient derived primary GBM cells have varying invading capabilities *in vitro* and *in vivo***
sECM encapsulated SC in the resection cavity track invading GBM cells in the brain
Targeting Cell Proliferation and Death Pathways in cancer cells
Both HDAC inhibitor and Cisplatin sensitize TRAIL resistant GBMs to TRAIL mediated apoptosis

Bagci-Onder et al Oncogene. 2012

Redjal et al Stem Cells. 2014
EGFR specific nanobodies for targeted EGFR tumor therapy

- Conventional monoclonal antibodies have limited success due their high molecular weight, preventing their delivery across the intact or partially disrupted blood-brain barrier and their physical diffusion into the tissues.

- Camels and llamas possess fully functional antibodies that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3).

- Nanobodies have:
  - high target specificity
  - low inherent toxicity
  - readily access receptor clefts and are extremely stable
  - have a low immunogenic potential
Characterizing EGFR specific Nanobodies

LV-ENb1

LV-Enb2

Nanobody expression (fold Day 7)

EGF (50 ng/ml): + + + + + + + + + + + +

Nanobody concentration (nM):

ENb2

ENb1

Nanobody expression (fold Day 7)

EGFR pY^{1068}

EGFR

Akt pSer^{473}

Akt

pMAPK p44/42

MAPK p44/42
TRAIL variant of EGFR nanobodies simultaneously targets EGFR and death receptors on tumor cells.
Blocking EGFR with Cetuximab significantly reduces ENb-TRAIL mediated apoptosis.
TRAIL variant of EGFR specific nanobodies has anti-tumor effect

**Graphs showing:**
- Tumor volumes (Fluorescence intensity x1000) for NSC-GFP, NSC-ENb, and NSC-ENb2-TRAIL with p-values of 0.05 and 0.02.
- Percent survival over days elapsed for Control, ENb2, and ENb2-TRAIL.
- Number of cleaved Caspase-3+ve cells for NSC-GFP, NSC-ENb, and NSC-ENb2-TRAIL.
Brain metastases are most common in patients with lung cancers, breast cancers and melanoma.

- 20-40% of patients with metastatic breast cancer develop brain metastasis.
- Majority of patients exhibit multiple tumors at the time of metastasis diagnosis.
Developing and Characterizing an imageable metastatic brain tumor model

Fluc bioluminescence in vivo
[photons/5min (x 10^6)]

Days after implantation

Inject MDA-Br2ma cells via intracarotid artery

Fluc imaging/histology

mCherry
CD31
Merged

Parenchymal metastatic foci
Leptomeningeal metastasis
Vascular co-option

Developing and Characterizing an imageable metastatic brain tumor model

mCherry
CD31
Merged

Vascular co-option

Parenchymal metastatic foci
Leptomeningeal metastasis
Therapeutic SC-TRAIL eradicate breast metastatic tumors in the brain

Expression of serpins, protects metastatic cancer cells from death signals and allow for efficient vascular co-option and colonization of tumor cells in the brain (Valiente et al., 2014).

mCherry- metastatic tumor foci
GFP- engineered stem cells

BRAIN
A JOURNAL OF NEUROLOGY

Targeting breast to brain metastatic tumours with death receptor ligand expressing therapeutic stem cells
Tugba Bagci-Onol, Wanlu Du, Jose-Luis Figueiredo, Jordi Martinez-Quintanilla and Khalid Shuh
Oncolytic Virus Therapy

Diagram:
- Normal cell
  - Viral replication inhibited
  - Normal cell spared
- Viral agent
  - Cancer cell (genetic target)
    - Viral replication proceeds
  - Tumor lysis—virus spread
Screening for oHSV and TRAIL resistant GBM cells

Established glioma cell lines

Primary glioma stem cell lines

S-TRAIL (ng/ml)

% viability (Fold control)

Caspase 3/7 activity (Fold control)

% viability (Fold control)

Virus yield (pfu/ml)

control

MOI 0.2

MOI 1
oHSV-TRAIL downregulates ERK and upregulates JNK and P38 and primes oHSV and TRAIL resistant GBM to Caspase mediated apoptosis

Tamura et al Oncogene. 2012
MSC loaded with oHSV allow replication and release of viral particles \textit{in vitro} and \textit{in vivo}
Fluorescence imaging reveals the dynamics of MSC-oHSV mediated infection of GBM cells *in vivo*.
sECM encapsulated SC-oHSV have anti-tumor efficacy in mouse models of GBM resection

Time course of oHSV spread in the resection cavity

- oHSV-Fluc
- MSC-oHSV-Fluc

Fluc signal intensity vs. Time post-resection (days)

Percent survival vs. Days post-implantation

Stem Cells Loaded With Multimechanistic Oncolytic Herpes Simplex Virus Variants for Brain Tumor Therapy

Matthias Dueben, Jordi Martinez-Quantanilla, Koushi Tamura, Shawn Hingtgen, Navid Redjai, Hiroaki Wakimoto, Khalid Shah

Manuscript received July 22, 2013; revised March 4, 2014; accepted March 7, 2014.

Correspondence to: Khalid Shah, MS, PhD, Molecular Neurotherapy and Imaging Laboratory, Massachusetts General Hospital, MGH East 149, 13th street, Charlestown, MA 02129 (e-mail: kshah@mhg.harvard.edu)
Developing tumor models that mimic the clinical scenarios are critical to developing efficacious treatments for GBMs.

The incorporation of mechanism based biological therapies is important for the advancement of the field.

Stem cells have the potential to be used as a therapeutic “protein factories” that can molecularly target proliferating GBM cells.

Non-invasive real time imaging provides key information on fate of GBM and therapeutic efficacy of stem cells.