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DUKE ENGINEERING

Outrageously Ambitious

Ravi V Bellamkonda
Vinik Dean of Engineering & Professor of Biomedical Engineering
Engineering strategies for a moving target – Invasive Gliomas

Ravi V Bellamkonda
Vinik Dean of Engineering & Professor of Biomedical Engineering (& Drs. Anjana Jain, Dr. Nassir Mokarram, Dr. Jonathan Lyon & Dr. Nalini Mehta)
Brain tumors are the most common solid tumors in children.
Invasive tumors are have the worst prognosis.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>20-44</th>
<th>45-54</th>
<th>55-64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade (diffuse) astrocytoma</td>
<td>59%</td>
<td>40%</td>
<td>NA</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>49%</td>
<td>29%</td>
<td>8%</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>16%</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>85%</td>
<td>77%</td>
<td>65%</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
<td>66%</td>
<td>53%</td>
<td>33%</td>
</tr>
<tr>
<td>Ependymoma/anaplastic ependymoma</td>
<td>99%</td>
<td>85%</td>
<td>84%</td>
</tr>
</tbody>
</table>
Engineering strategies for a moving target – dancing with invasion...

- Anti-invasion IB Nanocarriers (Sci Trans Med)
- Tumor Monorail
- Tumor encapsulation
- Electrotaxis of brain tumors
- Engineering motile nanocarriers for a moving target
- Tumor tractor beam
Invasion of glioblastoma often tracks along white matter tracts...

Pathology Image Database

Warning: “Only an engineer can think this” thought 😊

If gliomas are so invasive, is there a way we can exploit this?

Tumor monorail Project for Tumor ‘Exvasion’
Conceptually...
A Novel Brain Tumor ‘Exvasion’ Device

1. Aligned nanofiber film - promote cell migration
2. Apoptotic Hydrogel ‘Sink’ - Induce cell death
Topographical Influence on Tumor Cell Migration – PCL nanofiber films

Distance migrated (μm)

Day 1  Day 2  Day 4  Day 6  Day 8  Day 10

0  1000  2000  3000  4000  5000

Aligned Nanofibers

Smooth Film
Component 2: Hydrogel Sink - Cyclopamine-Apoptosis Inducing Drug

- Sonic hedgehog pathway over-expressed in high grade brain tumors
- Cyclopamine inhibits cell growth
Conjugate a drug, that induces cell programmed death, to a hydrogel scaffold

Conjugated Cyclopamine and Collagen
Migration of U87mg to Cytotoxic Cyclopamine Hydrogel
In vivo implementation with a PCL/PU tumor extraction device (with a built-in topography control)
U87mg Cells Migrate into the Entire Conduits with Nanofiber Films
Analyzing tumor load

- **IC**: Inside Conduit >3mm from base of conduit.
- **NE**: Near Exit, 0-800µm from base of conduit.
- **IT**: Inside Tumor, >800µm from base of conduit.
- Empty Conduit vs. Conduit with Aligned Nano-film down the middle.
Analyzing tumor volume in compartments…
Proliferation Rate Near Exit

- NE: Near Exit, 0-800μm from base of conduit.

Proliferation Rate Inside Tumor

- IT: Inside Tumor, >800μm from base of conduit.

Proliferation Rate All Areas

- All 3 Areas: IC, NE & IT
Possibly the first demonstration of:

- engineered, and controlled tumor migration in vivo
- Controlled migration of tumor into a cytotoxic hydrogel ‘sink’
- Bringing tumor to the drug rather than....
We have since replicated this study and are now analyzing cellular contents of the monorail device, effect of different materials etc.
Where are we going with this?

Reservoir/Sampling device

Tumor encapsulation project – Dr. Tarun Saxena
Our monorail device is still invasive..What about DIPG?
Investigating Electrotaxis of Glioblastoma Spheroidal Aggregates

Could we influence tumor invasiveness non-invasively?

Dr. Johnathan Lyon
Existing regimes of cancer electrotherapy

Adapted from Marjanovič & Miklavčič, Življenje in tehnika 2011.
Population-Level Electrotaxis

(a) Diagram showing the components of the setup:
- Front View: PDMS Cover, 200 μm, 5 mm, 19 mm, Agar-Salt Bridge, Media Reservoir
- Top View: Plating Region, 2-Sided Tape, Note: not drawn to scale

(b) Photograph of the actual setup with multiple electrodes and media reservoirs.

(c) Close-up image of a media reservoir.

(d) Close-up image of a U-shaped object.
Population-Level Electrotaxis

[Diagram showing centrifugation followed by 24h process]

Scale: 100 um
Population-Level Electrotaxis

Scale: 150 um

Pseudo-colored for time

RED: 0h
GREEN: 8h
U87MG Cells electrotax **cathodally** as spheroidal aggregates
Population-Level Electrotaxis

The diagram illustrates the cathodal bias in various cell lines under different conditions. The x-axis represents different cell lines: U87mg, LN229, A172, T98g, U373, DAOY, and D283Med. The y-axis shows cathodal bias in micrometers (µm) with control conditions represented by open bars and 250 V/m conditions represented by filled bars. The error bars indicate the variability of the measurements.
John has a detailed transcriptomic and inhibition analysis looking into mechanisms

IGF/PI3K/mTOR/AKT PATHWAY – U87MG

With inhibition of mTOR, mTORC1/2, or AKT1/2/3, all signs of electrotactic response are gone.

Inhibition of IGF1R/InsR had no effect, but was expected given no effect with PI3K(a/B/d).
Inevitably some invasive tumor nodules ‘escape’ – can we design strategies to treat dispersed tumors in the brain?

Dr. Nalini Mehta

Nalini Mehta, Johnathan G. Lyon, Ketki Patil, Nassir Mokarram, Christine Kim, Ravi V. Bellamkonda (2016) Bacterial Carriers for Glioblastoma Therapy, Molecular Therapy – Oncolytics
Our ability to treat spread tumors is very limited

- Nanocarriers are inherently diffusion limited
- There are two possible strategies
  - A magical drug that only reaches tumors and not any other ‘normal’ tissue
  - A drug/therapy that reaches everywhere but is only active at the tumor site
VNP20009 strain of ST: - $msbB^-$ and $purI^-$

Salmonella typhimurium
Carrier Design

Salmonella typhimurium
  • Strain: VNP20009*
  • Avirulent
  • localizes to tumors

Drug Production
  • Wild-type p53
  • Azurin

Targeting redundancy
  • Hypoxic Promoter

Tumor Inoculation → Tumor Formation → Salmonella Injection (1st) Day 7 → Salmonella Injection (2nd) Day 14
No survival benefit with carriers that express either Azurin or p53 alone
Kaplan-Meier Plot and Tumor Volume with combined p53 and Azurin

3 groups- Control, Non-responders, Responders
Absence of carrier-mediated systemic toxicity
Why do only 20% respond?

- It was an aggressive tumor
- There is evidence of apoptosis in the treated non-responders – need to play with ‘dosing’
- Are some tumors more susceptible than others – variability in tumors even when ‘seeded’ into athymic rats
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Questions?

- National Institutes of Health (EUREKA Award from NCI)
- Ian’s Friends Foundation, Atlanta
- Billi and Bernie Marcus Foundation
- Children’s Healthcare of Atlanta
- Mike and Denise Salvino