Biology and Therapeutic Implications of Tumor Dormancy

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Current lab members:

Technicians:  David Dales, Carl Postenka, Nicole Hague, Joseph Andrews, Carmen Simedrea

Graduate students:  Jenn MacLean, Michael Lizardo, Lesley Souter, Jen Mutrie, Caroline Whiston, Connor MacMillan

Research Associate:  Pieter Anborgh

Past lab members…including:  Keith Luzzi, Dianne Cameron, George Naumov, Sharon Vantyghem, Ben Hedley, Patricia McGowan, Brigitte Goulet, Jason Townson

Clinical colleagues who have influenced my thinking about dormancy:

Ted Vandenberg
London Regional Cancer Program

George Sledge
Indiana University Simon Cancer Center
Indianapolis

Paul Goss
Massachusetts General Hospital Cancer Center
Boston
Estimates for US 2010

• 209,060 new cases of breast cancer
• 40,230 breast cancer deaths
• Most of these deaths will be due to metastasis

>> Reduction in breast cancer mortality & morbidity requires better understanding of metastasis

>> New therapies should be assessed for ability to prevent, delay or treat metastatic disease

Cancer Statistics, 2010
Jemal et al., CA Cancer J Clin 60: 277
Metastatic inefficiency

Many circulating cancer cells → Few metastases

What steps in the metastatic process are responsible for metastatic inefficiency?
Cell accounting: 10 µm microspheres to quantify cell survival and metastatic inefficiency in mouse metastasis models

Need to know both numerator AND denominator:
Cells still present / Cells that originally arrived in the organ

Chambers et al., Breast Cancer Res 2: 400-407, 2000
Even highly metastatic cell lines are very inefficient. More cells remain dormant than form metastases.

<table>
<thead>
<tr>
<th>Time (p.i.)</th>
<th>Solitary Cells (%)</th>
<th>Micrometastases (4-16 cells)</th>
<th>Tumors (cells)</th>
<th>Total Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
<td>Inject 3x10^5 B16F1 cells</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>90 min</td>
<td>87.4</td>
<td>Subset of cells initiate growth</td>
<td></td>
<td>12.6</td>
</tr>
<tr>
<td>3 d</td>
<td>81.4</td>
<td>Subset of micrometastases continue growth</td>
<td></td>
<td>16.6</td>
</tr>
<tr>
<td>13 d</td>
<td>36.1</td>
<td>Persistence of dormant solitary cells</td>
<td></td>
<td>63.8</td>
</tr>
</tbody>
</table>

also Cameron et al, Cancer Res 60: 2541-2546, 2000
Townson et al., Cancer Res 69: 8326-31, 2009
How do highly and poorly metastatic populations differ?

- **B16F1 / liver**
- **B16F10 / lung**
  - Cameron, Cancer Res, 2000
- **NIH3T3 +/- ras / liver**
  - Varghese, Cancer Res, 2002
- **D2A1, D2.OR / liver**
  - Naumov, Cancer Res, 2002
- **MDA-MB-231 vs. 231BR / brain**

**Subset of cells responsible for metastasis**


McGowan et al., Molecular Cancer Research, in press
The Metastatic Process

- Cancer cells shed from 1º tumor travel via the circulatory system and arrest in vasculature at 2º sites by size restriction

- Tumor cells in 2º sites:
  - Dormant cells
  - Pre-angiogenic ‘dormant’ micrometastases
  - Vascularized metastases

Metastases form from a subset of cells – many more cells may remain dormant

Chambers et al. Nature Reviews Cancer 2002
Cytotoxic chemotherapy inhibits metastatic growth but does not reduce numbers of solitary dormant cells

**Experimental metastasis assay (D2.A1 mammary carcinoma cells)**
**Identification of dormant cells by retention of fluorescent nanospheres**
‘Cell accounting’

Use of Magnetic Resonance Imaging to follow fate of breast cancer cells metastasizing to brain

- MDA-MB-231BR human breast cancer cells form brain metastases after intracardiac injection in mice
  
  Yoneda et al., J Bone Miner Res 16; 1486-1495, 2001

- MDA-MB-231BR/EGFP cells – green fluorescent protein
  
  Patricia Steeg, Diane Palmieri, Julie Bronder

- MDA-MB-231BR/EGFP cells labeled in vitro with MPIO
  
  Paula Foster, Brian Rutt, Chris Heyn, Ann Chambers
  Micron-sized Iron Oxide Particles – taken up by cells in culture – detectable as signal voids in MRI
  Retained by cells until diluted by cell division

Funded by DoD - Patricia Steeg’s Center of Excellence on Brain Metastasis of Breast Cancer
Single Cell MRI

- Clinical field strength (1.5 - 3T)
- High strength gradient insert
- Custom-made RF coils
- FIESTA pulse sequence

Townson J et al., Cancer Research 69: 8326-31, 2009
In vivo MRI to Monitor Fate of Breast Cancer Metastases in Mouse Brain – 4D metastasis assay

MDA-MB-231BR human breast cancer cells (GFP)

Iron-loaded before injection (MPIO)

Intracardiac injection in mice

MRI every 1 - 4 days – whole brain

Clinical 1.5 T MR scanner with custom mouse coil – FIESTA pulse sequence

Single cells = MR signal voids (dark)

Metastases = MR hyperintensity (light) and GFP on histology

Fate of 231BR cells in mouse brain over 28 days after intracardiac injection - MRI

Red: Signal voids – iron-retaining cells – non-proliferating

Green: Signal hyperintensities – growing metastasis

Fate of 231BR cells in mouse brain 28 days after intracardiac injection

<table>
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<th>Day 28 MRI Void volume (mm³)</th>
<th>% of day 0 MRI Void volume (mm³)</th>
</tr>
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<tr>
<td>‘Transient’ cells</td>
<td>33.5 ± 3.3</td>
<td>93.9 ± 0.7</td>
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<tr>
<td>Non-proliferating cells</td>
<td>1.6 ± 0.13</td>
<td>4.5 ± 0.8</td>
</tr>
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<td>Proliferating cells</td>
<td>0.56 ± 0.07</td>
<td>1.6 ± 0.06</td>
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* Are (all? some of?) these dormant cells that can re-awaken? How kill them? (Would that matter clinically?)

Townson J et al., Cancer Research 69: 8326-31, 2009
Tumor dormancy

**Clinically**
- Apparently successful treatment of primary tumor
- Subsequent tumor recurrence (local, metastatic)
- Dormancy can be long (e.g., breast cancer, melanoma, 20-25 yr)
- Years of uncertainty for patients / clinical management?
- “Dormancy” vs “cured”?? (plateau vs. declining survival curves; autopsy studies?)
- How assign risk? Does adjuvant therapy help?
- *Are dormant cells an appropriate clinical therapeutic target?*

**Biologically**
- Status of tumor cells during clinical dormancy is poorly understood
- Dormant cells observed in experimental metastasis models
Does tumour dormancy offer a therapeutic target?

Paul E. Goss and Ann F. Chambers

Abstract | The increasing number of cancer survivors is cause for celebration, but this expanding population has highlighted the problem of tumour dormancy, which can lead to relapse. As we start to understand more about the biology of dormant cancer cells, we can begin to address how best to treat this form of disease. Preclinical models and initial clinical trials, as exemplified in patients with breast cancer, are paving the way to address how best to treat long-term cancer survivors to minimize the risk of cancer recurrence.
Many cancer types are at risk for late recurrence.

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>5 years‡</th>
<th>10 years§</th>
<th>15 years‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (female)</td>
<td>90%</td>
<td>82%</td>
<td>75%</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>93%</td>
<td>90%</td>
<td>89%</td>
</tr>
<tr>
<td>Kidney and renal pelvis</td>
<td>69%</td>
<td>61%</td>
<td>54%</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>68%</td>
<td>58%</td>
<td>54%</td>
</tr>
<tr>
<td>Lung and bronchus</td>
<td>16%</td>
<td>11%</td>
<td>9%</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>19%</td>
<td>12%</td>
<td>9%</td>
</tr>
<tr>
<td>Liver and intrahepatic bile duct</td>
<td>14%</td>
<td>9%</td>
<td>8%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6%</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Stomach</td>
<td>27%</td>
<td>21%</td>
<td>18%</td>
</tr>
<tr>
<td>Brain and other nervous system</td>
<td>36%</td>
<td>32%</td>
<td>30%</td>
</tr>
<tr>
<td>Oral cavity and pharynx</td>
<td>63%</td>
<td>51%</td>
<td>43%</td>
</tr>
<tr>
<td>Larynx</td>
<td>63%</td>
<td>49%</td>
<td>41%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>82%</td>
<td>75%</td>
<td>71%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>97%</td>
<td>97%</td>
<td>96%</td>
</tr>
<tr>
<td>Ovary</td>
<td>46%</td>
<td>38%</td>
<td>38%</td>
</tr>
<tr>
<td>Prostate</td>
<td>100%</td>
<td>95%</td>
<td>82%</td>
</tr>
<tr>
<td>Testis</td>
<td>96%</td>
<td>95%</td>
<td>94%</td>
</tr>
<tr>
<td>Corpus and uterus, NOS</td>
<td>84%</td>
<td>80%</td>
<td>77%</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>72%</td>
<td>67%</td>
<td>65%</td>
</tr>
</tbody>
</table>

NOS, not otherwise specified. Table is based on Surveillance, Epidemiology and End Results (SEER) data (see Further information), which was provided by the American Cancer Society. ‡Cases diagnosed from 1999 to 2005 and followed during 2006. §Cases diagnosed from 1994 to 2006 and followed during 2007. ‖Cases diagnosed from 1989 to 2006 and followed during 2007.
Long-Term Risk of Breast Cancer Recurrence: Two Cell Populations?

SLIDE COURTESY OF GEORGE SLEDGE

![Graph showing recurrence hazard rate vs. years for two cell populations: Proliferating Micromets and Relapsing Dormant Cells.]

ER = estrogen receptor
PgR = progesterone receptor

Long-term therapy is being used with success in hormone-responsive breast cancer

Tamoxifen $n = 5187$

Placebo $n = 2594$

Letrozole $n = 2593$

Letrozole (LET) $n = 2457$

No Letrozole (PLAC) $n = 613$

Letrozole (PLAC-LET) $n = 1655$

Median follow up

30 Months

54 (16 – 86) Months

1998

2003

2005

Unblinding

Ingle et al

Goss et al
Long-term therapy (Letrozole) does prevent recurrences ...

... but at a cost of some adverse events

There is a persistent (but small) risk of recurrence

MA.17 clinical trial
Much to learn …

How common are dormant cells (autopsy studies, imaging studies)?
Where are they? How likely are they to ‘re-awaken’?
What causes them to ‘re-awaken’? Who should/should not be treated?
What regulates metastatic growth vs. dormancy – microenvironment?
How to balance benefits vs. toxicities of long-term therapy?

Goss and Chambers, Nature Reviews Cancer 2010