The search for intestinal stem cells: lessons learned

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NASPGHAN State of the Art lecture
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Outline

• Review historical literature relating to intestinal stem cells (ISCs)
• Explain the challenges facing isolation of ISCs
• Current understanding of ISC populations
  – Homeostasis
  – Challenge e.g. after damage
• Present our new data on ISC fractions isolated by side population (SP) sorting

Growth in the ISC Field
1974 Cheng H and Leblond CP

Key Contributions from Potten Lab

First Transcript Analysis of ISCs

Table 1: Location of CBCs

<table>
<thead>
<tr>
<th>Cell position</th>
<th># of CBCs with phagosomes</th>
</tr>
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<tbody>
<tr>
<td>1–3</td>
<td>20</td>
</tr>
<tr>
<td>4–6</td>
<td>30</td>
</tr>
<tr>
<td>7–9</td>
<td>9</td>
</tr>
<tr>
<td>10–12</td>
<td>1</td>
</tr>
<tr>
<td>13 and higher</td>
<td>0</td>
</tr>
</tbody>
</table>
Growth in the ISC Field

- 2004: Bmp as "brake" vs. Wnt as "driver" (He et al.)
- 2003: First marker reported: Msi-1 (Potten et al., Kayahara et al.)
- 2005: First isolation of an ISC-enriched fraction (Dekaney et al.)
- 2007: Identification of Lgr5 as a marker of CBC (Barker et al.)

Identification of Lgr5 as a Marker of ISCs

Barker et al. 2007

- Lgr5 initially identified as Wnt target gene
- ISH showed expression restricted to CBC
- Lineage tracing demonstrated:

2009 Successful ISC Culture

- Ootani et al.:
  - Minced tissue in collagen
  - Air-liquid interface
  - Myofibroblast-dependent

- Sato et al.:
  - Single Lgr5-EGFP cells
  - In Matrigel
  - Added R-spo, Jagged, Noggin, EGF
Current understanding: ISC Subtypes

- **Actively cycling**
  - Lgr5
  - Olfm4
  - Ascl2
  - Sox9

- **Slow or non-cycling**
  - DNA-LRC
  - mTert
  - Bmi1
  - Hopx
  - Lrig1
  - Dclk1
  - H2B-LRC

Which matters: Homeostasis?

- **Barker et al. 2007**
  - Lgr5 cells actively cycling
  - Lineage tracing

- **Tian et al. 2011**
  - Ablated Lgr5 cells - homeostasis unaffected
  - Lineage tracing from Bmi1 increases

Conclusion: Intestine highly adaptable

Question: Role of other +4 ISC?

Which matters: Repair after Damage?

**Role of CBC-ISC**

- **Hua et al. 2012**
  - Lgr5 cells reduced but number surviving predicts crypt recovery

- **Van Landeghem et al. 2012**
  - Sox9<sup>low</sup> cells
    - Normally 24% Edu<sup>+</sup>
    - After irradiation 63% Edu<sup>+</sup>
Which matters: Repair after Damage?

<table>
<thead>
<tr>
<th>Role of +4-ISC</th>
<th>DNA-LRCs: Potten et al. 1978</th>
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<tbody>
<tr>
<td></td>
<td>Control = none</td>
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<tr>
<td></td>
<td>Radiation LRC = 10% +4 cells</td>
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</table>

DCAMKL: Dclk1: May et al. 2007

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<tr>
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<td>Proliferation induced by radiation</td>
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mTert: Montgomery et al. 2011

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<tbody>
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<td>Lineage tracing increases markedly</td>
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Bmi1: Ton et al. 2012

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<td>Proliferate and tracing increases</td>
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Lrig1: Powell et al. 2012

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Intestinal Regeneration- Remarkable Redundance/Adaptability

![Intestinal Regeneration Diagram](Image)

Our Goals

- To devise sorting strategies to isolate ISC from WT mice
- Thus applicable to human tissue

Approaches

- Side population sorting (Dekaney et al. 2005)
- Membrane markers (CD24, von Furstenberg et al. 2012)
Side Population (SP) Sorting

- Originally described to isolate hematopoietic stem cells
  Goodell et al. 1996
- Subsequently applied to stem cells of several tissues
- Relies on ability of stem cells to efflux Hoechst dye – blocked by verapamil
- Intestinal SP enriched in Msi1
  Dekaney et al. 2005
- Comprises 1% total epithelium
- Microarray showed de-enriched for mitosis/cell cycle
  Gulati et al. 2008

<table>
<thead>
<tr>
<th>SP</th>
<th>SP (fold inc)</th>
<th>% Lgr5+ cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>17%</td>
<td>17</td>
<td>9</td>
<td>Unchanged</td>
</tr>
<tr>
<td>14%</td>
<td>14</td>
<td>21</td>
<td>Unchanged</td>
</tr>
<tr>
<td>13%</td>
<td>7</td>
<td>9</td>
<td>Unchanged</td>
</tr>
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**Conclusion:**
- % Lgr5+ cells does not track with crypt fission
- SP represents a different ISC population

Where would active ISCs fit into SP?

- Bone marrow – actively cycling population above traditional SP

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<th>New intestinal SP subfractions</th>
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<tr>
<td>USP</td>
<td>LSP</td>
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Hypothesis: SP analysis will distinguish active vs quiescent intestinal stem cells

Where are the actively cycling cells?

Identification of proliferating cells
• EdU given 1 hr before tissue collection
• Upper and Lower SP collected
• Then analyzed for EdU

Lgr5-EGFP\textsuperscript{hi} cell tracking expt
qRT-PCR of Upper SP

Fold change vs Intact Jejunum

The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent +4 cell markers

Muñoz et al. 2012, EMBO

qRT-PCR of Lower SP

Fold change vs Intact Jejunum

Quantifying EE in SP fractions

Method:
1. Collect USP and LSP by cell sorting
2. Label with synaptophysin antibody (pan-EE marker) - Bjerknes and Cheng (2010)
3. Re-analyze by flow cytometry for synaptophysin positive cells

Upper SP

Lower SP

0.3 ± 0.09% 1.8 ± 0.5%

Synaptophysin-DL640
Conclusions

**Upper SP**
- EdU data show rapidly dividing cells
- Lgr5-EGFP® almost exclusively tracks to Upper SP
- Further studies needed to assess purity

**Lower SP**
- EdU shows this is non-cycling in vivo
- Activated *in vitro* and shows ISC behavior
- Enriched in quiescent ISC transcripts
- Absence of active ISC markers and most lineages
- Traces of EE cells (1.8%)

Significance

- Novel, non-reporter based, method applicable to any mouse and readily translatable to human
- Allows for simultaneous isolation or examination of:
  - Active ISCs
  - Quiescent ISCs
- Lower SP is particularly interesting
Significance of Lower SP

- Captures multiple quiescent ISC populations
- Numbers predict rates of crypt fission
- Valuable tool for assessing responses to damage

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