The search for intestinal stem cells: lessons learned

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NASPGHAN State of the Art lecture

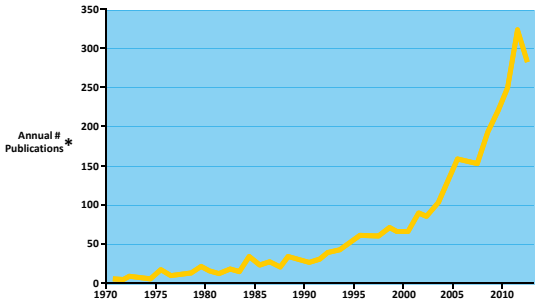
October 2013

UNC
Gastrointestinal Stem Cell Group

Outline

- Review historical literature relating to intestinal stem cells (ISCs)
- Explain the challenges facing isolation of ISCs
- Current understanding of ISC populations
- Summarize literature of "which matters"
  - 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

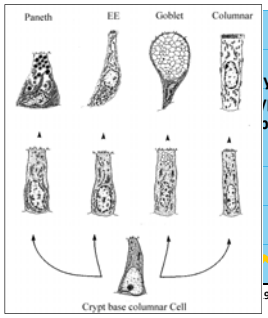


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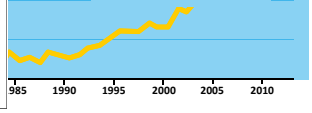
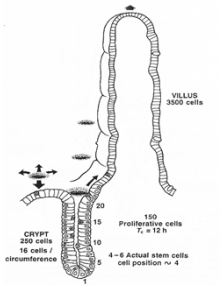
Table 1: Location of CBCs

Cell position	# of CBCs with phagosomes
1 – 3	20
4 – 6	30
7 – 9	9
10 – 12	1
13 and higher	0

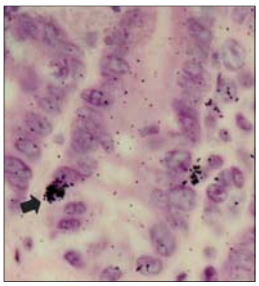


Key Contributions from Potten Lab

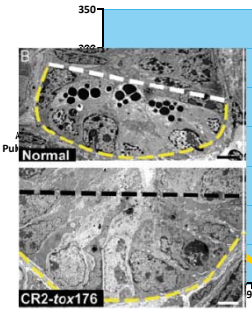
1998
Cell Numbers and Kinetics

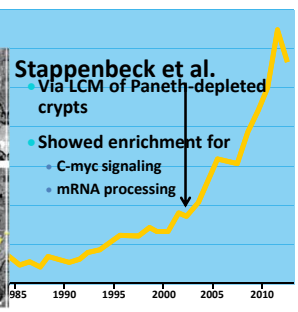


2002
Identification of label retaining cells



First Transcript Analysis of ISCs

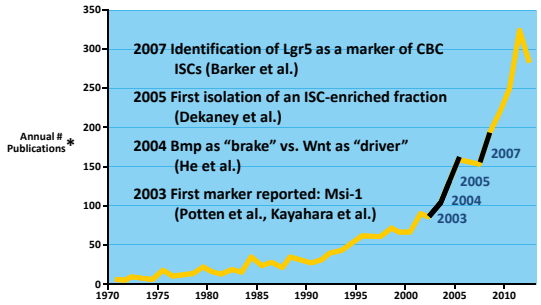




Stappenbeck et al.

- Via LCM of Paneth-depleted crypts
- Showed enrichment for
  - C-myc signaling
  - mRNA processing

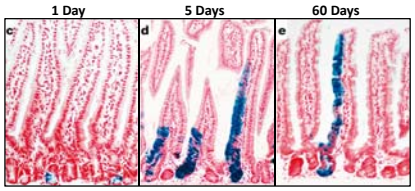
Growth in the ISC Field



Identification of Lgr5 as a Marker of ISCs

Barker et al. 2007

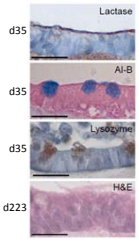
- Lgr5 initially identified as Wnt target gene
- ISH showed expression restricted to CBC
- Lineage tracing demonstrated:



2009 Successful ISC Culture

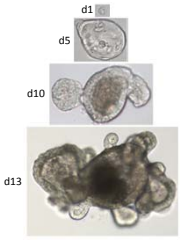
Ootani et al.

- Minced tissue in collagen
- Air-liquid interface
- Myofibroblast-dependent

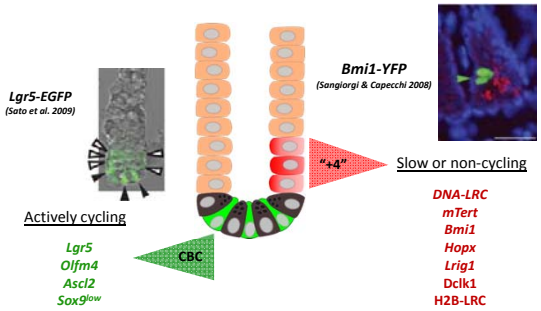


Sato et al.

- Single Lgr5-EGFP cells
- In Matrigel
- Added R-spo, Jagged, Noggin, EGF

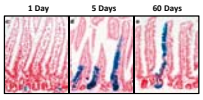


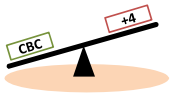
Current understanding: ISC Subtypes



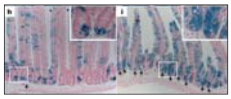
Which matters: Homeostasis?

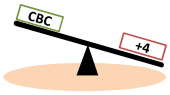
- *Barker et al. 2007*
  - Lgr5 cells actively cycling
  - Lineage tracing





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



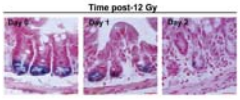


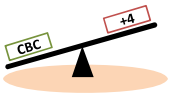
Conclusion: Intestine highly adaptable
Question: Role of other +4 ISC?

Which matters: Repair after Damage?

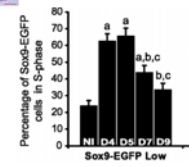
Role of CBC-ISC

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



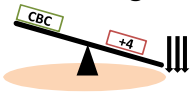


- *Van Landeghem et al. 2012*
  - Sox9^{low} cells
    - Normally 24% Edu^{+}
    - After irradiation 63% Edu^{+}



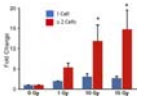
Which matters: Repair after Damage?

Role of +4-ISC



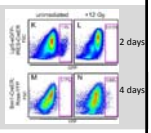
DNA-LRCs: *Potten et al. 1978*
- Control – none
- Radiation LRC – 10% +4 cells

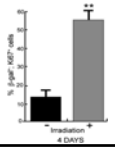
DCAMKL-1/Dclk1: *May et al. 2007*
- Proliferation induced by radiation



mTert: *Montgomery et al. 2011*
- Lineage tracing increases markedly

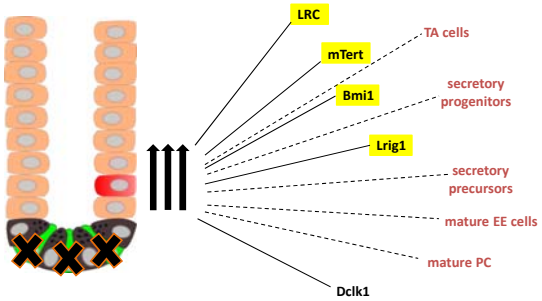
Bmi1: *Yan et al. 2012*
- Proliferate and tracing increases





Lrig1: *Powell et al. 2012*
- Proliferate and tracing increases

Intestinal Regeneration- Remarkable Redundance/Adaptability



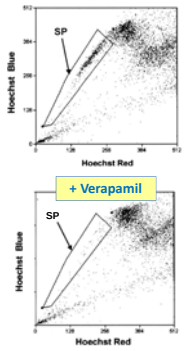
Our Goals

- 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. 2011*)

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
•Comprises 1% total epithelium
Dekaney et al. 2005

•Microarray showed de-enriched for mitosis/cell cycle
Gulati et al. 2008

Relationship between SP and Crypt Fission

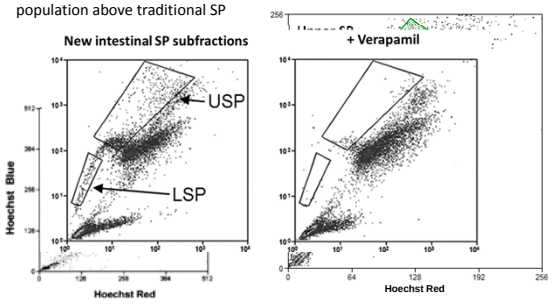
	Crypt Fission (%)	Crypt Fission (fold inc)	SP (fold inc)	% Lgr5⁺ cells	References
Resection	17%	17	9	----- Unchanged	*Dekaney, et al. (2007) Garrison, et al. (unpub.)*
Regeneration-doxorubicin	14%	14	21	Unchanged	*Dekaney, et al. (2009)*
Development	13%	7	9	Unchanged	*Dehmer, et al. (2011)*

Conclusion:

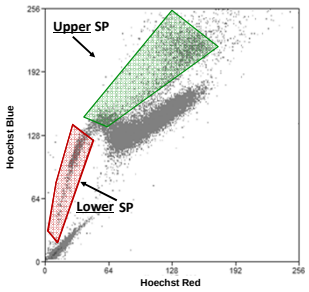
- % Lgr5⁺ cells does not track with crypt fission
- SP represents a different ISC population

Where would active ISCs fit into SP?

• Bone marrow – actively cycling population above traditional SP



Hypothesis: SP analysis will distinguish active vs quiescent intestinal stem cells



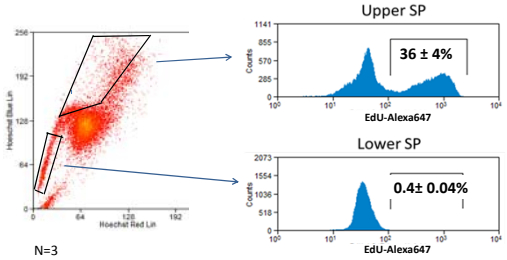
Upper SP
(actively cycling)

Lower SP (quiescent)
formerly our "SP"

Where are the actively cycling cells?

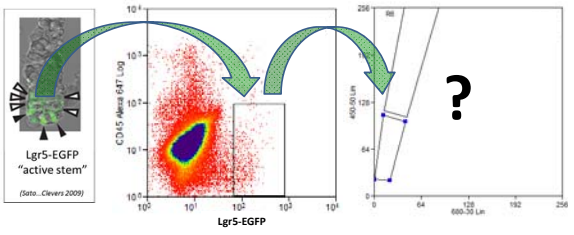
Identification of proliferating cells

- EdU given 1 hr before tissue collection
- Upper and Lower SP collected
- Then analyzed for EdU+



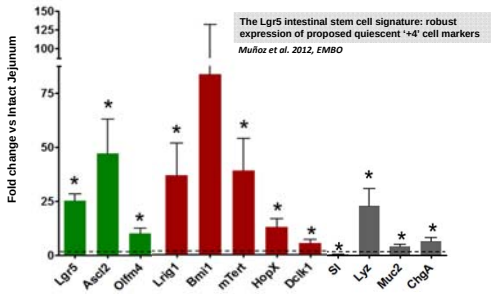
N=3

Lgr5-EGFP^{hi} cell tracking expt

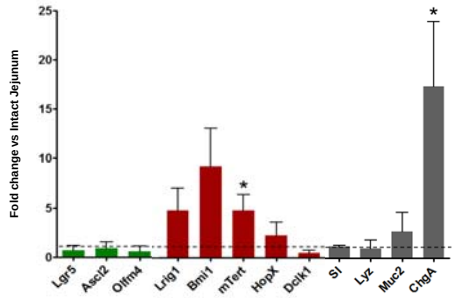


N=3

qRT-PCR of Upper SP



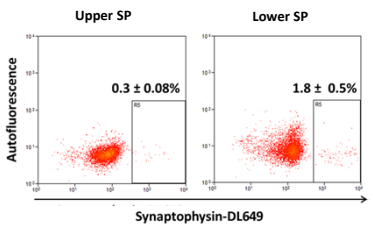
qRT-PCR of Lower SP



Quantifying EE in SP fractions

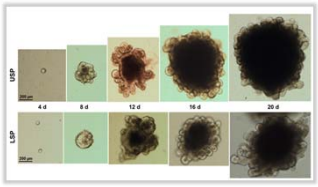
Method:

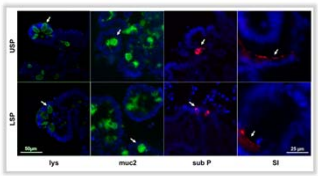
1. Collect USP and LSP by cell sorting
2. Label with synaptophysin antibody (pan-EE marker) - ***Bjerknes and Cheng (2010)***
3. Re-analyze by flow cytometry for synaptophysin positive cells



SP Subfractions in Matrigel Culture

- Modified Sato conditions
- Growth of actively cycling Upper SP was expected
- Non-cycling Lower SP result was surprising, environment shifts phenotype?
- Enteroids from both SP subfractions express markers of the 4 intestinal lineages





Conclusions

Upper SP
- EdU data show rapidly dividing cells
- Lgr5-EGFP^{hi} almost exclusively tracks to Upper SP
- Further studies needed to assess purity

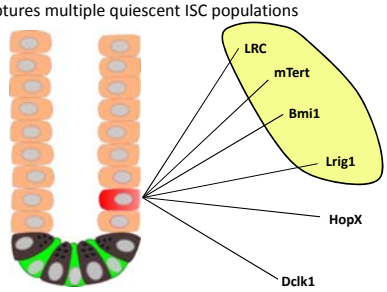
Lower SP
- EdU shows this is non-cycling *in vivo*
- Activated *in vitro* and shows ISC behavior
- Enriched in quiescent ISC transcripts
- Absence of active ISC markers and most lineages
- Traces of EE cells (1.8%)

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

Acknowledgements

Chris Dekaney
Michael Helmrath
Ajay Gulati
Aaron Garrison
Jeffrey Dehmer
Elizabeth Speck
Richard von Furstenberg
Brian Smith
Kristen Seiler
Erica Schenhals

R01 DK69585; U01 DK85547; T32 GM 008450; T32 DK007737; P30 DK34987

ISCC Intestinal Stem Cell Consortium

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UNC Core Labs
Flow cytometry - Nancy fisher
- Joan Kalnitsky
Histology - Kirk McNaughton
- Ashley Ezzel
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