Preview

Intestinal Stem Cell Dynamics: A Story of Mice and Humans

Michael C. Hodder[1, 3]

Dustin J. Flanagan[1, 3]

Owen J. Sansom[1, 2, *]

o.sansom@beatson.gla.ac.uk

[1]CRUK Beatson Institute, Glasgow G61 1BD, UK

[2]Institute of Cancer Sciences (ICS), University of Glasgow, Glasgow G61 1QH, UK

[*]Corresponding author

[3]These authors contributed equally

Stem cell dynamics define the probability of accumulating mutations within the intestinal epithelium. In this issue of *Cell Stem Cell*, Nicholson et al. (2018) report that human intestinal stem cell dynamics differ significantly from those of mice and establish that oncogenic mutations are more likely to expand; therefore, “normal” epithelium may carry multiple mutations.

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Main Text

The famous 1785 Robert Burns poem “To a Mouse” describes how a mouse carefully builds a nest in a wheat field only to have it destroyed by a farmer’s plough. Including the renowned phrase, “the best-laid plans of mice and men often go awry”; this poem draws parallels with a report from Nicholson et al. (2018, this issue of Cell Stem Cell) that human and mouse intestinal stem cell (ISC) dynamics differ substantially.

Investigation of ISC properties have provided insights into tissue homeostasis and disease. However, technical constraints largely prohibit direct measurement of these properties in humans, and as such, the mouse has served as a model organism to unveil the complexities of stem cell competition. Nicholson et al. (2018) report that human ISCs exhibit substantially different dynamics than those observed in mice and characterize examples of mutations that can distort ISC dynamics.

It is well established that intestinal crypts contain multiple ISCs. Mouse ISC studies show that they divide daily and compete randomly for limited niche space at the crypt base via the process of neutral drift (Vermeulen and Snippert, 2014). Neutral drift suggests that each functional ISC has an equal probability ($p = 0.5$) of replacing its neighbor, and thus over time, a crypt will drift toward monoclonality and become “fixed.” At the clonal level, stochastic competition between ISCs provides an opportunity for clonal progeny to expand or retract, either displacing the ISC-clones from the crypt or resulting in crypt fixation. Somatic mutations in genes such as *Apc* or *Kras*, or changes to the ISC niche such as reduced Wnt ligand secretion (Huels et al., 2018), alter competition between ISCs. Mutant ISCs can have a greater chance of displacing non-mutant ISCs, thus increasing the probability of mutant crypt fixation, a key requirement for pervasive cell transformation (Snippert et al., 2014; Vermeulen et al., 2013).

Nicholson et al. (2018) define baseline human ISC dynamics by looking for known spontaneous mutations that continuously label ISCs in the human colon. One such mutation is in the gene that encodes the enzyme O-acetyl transferase (OAT), the absence of which is visualized via mild Periodic acid-Schiff (mPAS) staining. Loss of O-acetylation of sialomucins in patient colonic epithelium ($mPAS^{+}$) was detected using automated image analyses. This revealed that the number of partially populated crypts (PPCs) remains constant over time in accordance with the theory of neutral drift, whereas the number of wholly populated crypts (WPCs) increased with age, suggesting that a PPC can

either become fixed or be replaced (Figure 1). Using this knowledge, the authors determine that the average time to monoclonality in a human colonic crypt is 6.3 years, a pedestrian pace when compared with mouse crypts, where monoclonality can be reached in a matter of weeks. A recent corroborating report uses mathematical modeling to infer that human colonic ISCs predominantly undergo asymmetric cell division, accounting for the leisurely pace of crypt fixation (Stamp et al., 2018).

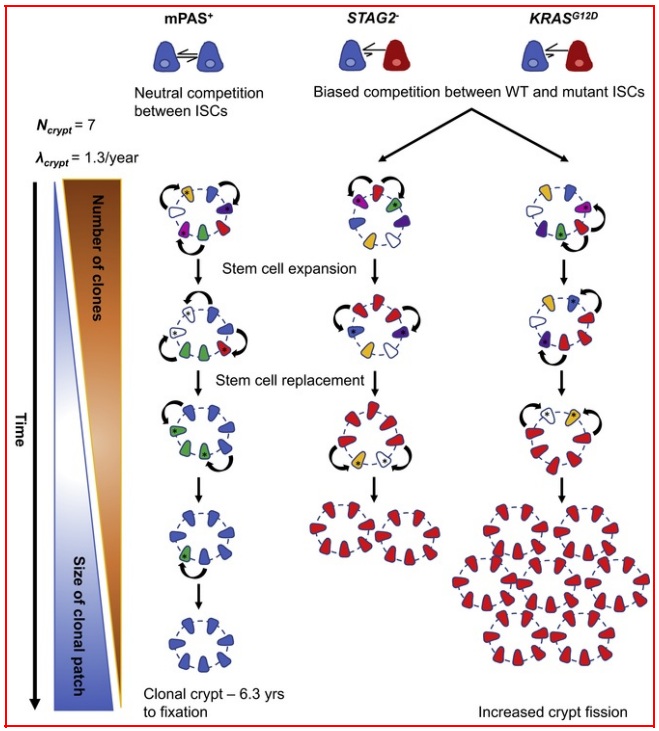

Figure 1 Somatic Mutations Can Bias Clonal Dynamics of Human Colonic Crypts

Cross-sectional schematic of human colonic crypts showing intestinal stem cell (ISC) clonal dynamics. In the presence of neutral somatic mutations (mPAS^{+}), all functional ISCs have an equal chance of expanding (curved arrows) or being replaced (*) from the crypt. Over time, the number of mutant functional ISCs (N_{crypt}) decreases as some are replaced by wild-type neighbors and lost. However, the mutant clones that remain trend toward clonal fixation, which occurs over many years. Pro-oncogenic mutations, such as *STAG2* or *KRAS*^{G12D}, bias ISC-competition and increase the frequency of crypt fission and clonal patch expansion.

To determine the *de novo* mutation rate, Nicholson et al. (2018) stained serial sections to identify single-cell clones and used this information to infer baseline stem cell behaviors. The authors conclude that human colonic crypts contain an average of 7 ISCs (N_{crypt}) that replace each other ~1.3 times/yr/crypt (λ_{crypt}), a rate that is approximately two orders of magnitude lower than that of mouse ISCs (Kozar et al., 2013). Other reports have calculated a comparable number of ISCs per crypt (Baker et al., 2014; Stamp et al., 2018).

Next, the authors identify somatic mutations that bias competition between ISCs. *STAG2*, which forms part of the cohesion complex and is a known tumor suppressor, confers a competitive advantage over non-mutant ISCs. The authors suggest the diminished ratio of WPC to PPC are indicative of increased competitive advantage, meaning that *STAG2* mutant clones are almost certain ($p = 0.99$) to outcompete their non-mutant counterparts. Critically, this is the first quantification of intra-cryptal mutation-driven bias in human colonic ISCs (Figure 1).

Intra-cryptal competition is just one way for mutations to achieve epithelial colonization. As such, the authors investigate crypt fission as an alternative means to permit mutant clone expansion. Basal crypt fission rates for neutral mutations were calculated as ~0.7%/yr. Interestingly, the crypt fission rate measured in *STAG2* mutant crypts was 3-fold higher (2.15%/yr) than that observed in neutral mutant crypts.

Previous reports have noted the presence of *KRAS* mutant crypts in otherwise morphologically normal human colon. However, the authors argue that without a substantial competitive advantage, *KRAS* mutations would go undetected because of the markedly low mutation rate. Using targeted sequencing to detect *KRAS* mutations, the authors conclude that intra-cryptal fixation cannot account for the quantity of *KRAS* mutant cells. Instead, the authors

propose that a 10-fold increase in *KRAS* mutant crypt fission can account for the observed biological dataset (Figure 1). This would result in large patches of *KRAS* mutant crypts in otherwise non-neoplastic tissue, confirming previous reports of increased crypt fission in *Kras* mutant mouse epithelium (Snippert et al., 2014).

Importantly, Nicholson et al. (2018) define basal metrics to describe stem-cell competition within the human colon. While this is not the first report of human colonic stem cell dynamics, it is the first to quantify the effect of advantageous mutations upon both intra-cryptal dynamics and crypt fission. Compared with neutral mutations, the authors conclude that over a lifetime, the pro-oncogenic mutations *KRAS* and *STAG2* have a 155-fold and 13-fold greater expansion coefficient, respectively.

The study from Nicholson et al. (2018) has implications for the ISC and cancer fields. For instance, the human colon, rather than being ubiquitously composed of non-mutant cells, is likely to be a mosaic of mutant clonal patches, which will shape the subsequent interpretation of mutant phenotypes. Similarly, founding mutations may influence the fitness of secondary mutations that occur within the same clonal patch. For example, clonal fixation of *APC* mutant cells may differ profoundly if a pre-existing *KRAS* mutation is present. This may potentiate rapid expansion of neoplastic clones, in line with recent reports describing tumor mutation hierarchy and the so-called “big bang” model (Roerink et al., 2018; Sottoriva et al., 2015). Therefore, the colon and other epithelia may mimic the skin, where multiple mutations are required to subvert homeostatic-constraint mechanisms. For example, we observed that loss of *Tgfbr1* drove rapid invasive carcinoma when combined with $Kras^{G12D}$, whereas $Kras^{G12D}$ alone had little phenotype and failed to drive cancer (Cammareri et al., 2016).

In the future, it will be interesting to assess if any common colorectal cancer risk alleles or lifestyle predispositions impinge on human-stem-cell dynamics. Moreover, given the sluggish pace of clonal fixation, this could provide a promising therapeutic window to eradicate premalignant clones prior to fixation.

Declaration of Interests

The authors declare no competing interests.

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