Quantifying human intestinal stem cell and crypt dynamics: the implications for cancer screening and prevention

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The basic functional units of the human colon are crypts – test-tube-shaped invaginations of the mucosa, lined with a single layer of epithelial cells. The cells of the crypt continuously and rapidly regenerate as a result of stem cell divisions at the crypt base. This gives rise to daughter cells, which then migrate along the crypt axis toward the lumen where they are shed. In recent years, mouse models wherein heritable fluorescent labels can be specifically induced within the stem cell population have been widely used to study the evolutionary dynamics of the stem cell pool [1–4]; however, in humans, we have until recently had little insight into how stem cell populations evolve. Critical parameters such as how many functional stem cells are present in each crypt, how long it takes a new stem cell clone to repopulate the crypt, and how often crypts divide have remained elusive. Understanding stem cell evolution is a critical step in developing chemopreventive strategies: if we know ‘the rules’ of how stem cells evolve, we can design strategies to optimally intervene in their evolutionary game. Crypt divisions drive tumor growth in the bowel, and so this parameter is critically relevant for understanding the timescale of neoplastic growth – which has direct relevance for endoscopic screening strategies.

**Human colonic stem cells evolve by neutral drift dynamics**

We recently applied a novel analysis of healthy and diseased human colon tissue, involving lineage labeling combined with mathematical analysis, to determine the dynamics of the human stem cell niche [5]. Historically, a critical limitation when analyzing stem cell dynamics in human samples has been the difficulty of sampling over time. In our analysis, we circumvented this problem by recognizing that the distance a cell has migrated along the crypt axis is proportional to the time elapsed since the cell was generated by a stem cell division at the crypt base. Therefore, if we were able to introduce a heritable label into a single stem cell at the crypt base, the temporal growth and contractions of this labeled clone at the crypt base could be read out along the crypt axis: the recent size of the stem cell clone is recorded by the size of the labeled clone at the crypt base, and progressively more historical sizes revealed closer to the lumen. In other words, along the length of each crypt, there is a short-term record of events that have occurred in the stem cell niche.

Clearly, introducing artificial heritable labels into human cells *in vivo* is not possible, so instead we used the activity of a mitochondrial enzyme (cytochrome c oxidase [CCO]) as a histochemically detectable label to detect clones. In the crypt, cells that acquire a somatic mutation in CCO lose activity of the enzyme and so are readily visualized histochemically as a ‘ribbon’ of CCO-deficient cells migrating from the crypt base. We mathematically analyzed the expansions and contractions of this ‘ribbon’ along the crypt axis, and reconstructed the events within the stem cell niche. We found that in the human colon, expansion of a stem cell population was perfectly balanced by contraction, indicating that the niche is governed by ‘neutral drift’ dynamics, an observation consistent with data from mouse models [2,3]. Furthermore, our analysis suggested that nearly all stem cell divisions were symmetric (producing either two daughter stem cells or no daughter stem cells), and this again serves to illustrate the inherent dynamic and plastic nature of the stem cell population. We note though that this conclusion was critically dependent on the imprecise measurement of the cell division rate at the crypt base.
We also investigated how mutations in the adeno-
matus polyposis coli (APC) gene – often described as
the key gatekeeper mutation in colorectal carcino-
genesis – altered stem cell evolution. We did this by study-
ing familial adenomatous polyposis (FAP) patients who
inherit one mutant copy of the tumor suppressor gene
APC (‘APC+/-’). Upon loss of the second copy of APC
(‘APC-/-’) the crypt becomes morphologically adeno-
matous, which is thought to be a prerequisite for
colorectal cancer (CRC). Interestingly, we found that
the loss of APC altered the dynamics of the stem cell
niche, with APC-/- crypts having an increased number
of functional stem cells, and also an increased loss/
replacement rate, but importantly both APC+/- and
APC-/- stem cell niches remain governed by neutral
drift dynamics.

This neutral drift behavior has important impli-
cations for understanding how tumorigenic mutations arise and
may persist in the colonic mucosa. First, we assume that
such a mutation is most likely to occur in a proliferative
stem cell. Recent data suggests that the ‘stemness’ is not a
cell-intrinsic property, but is instead governed by the
location occupied in the niche [4]. Therefore, stem cell
populations compete to occupy space in the niche, and
for a mutation to become fixed in the crypt, the mutant
lineage must take over the stem cell niche by excluding
wild-type stem cells. Our data shows that within a crypt’s
stem cell population, each cell has equal chance of being
lost from the niche, or growing to become dominant in the
niche. If we make the not unreasonable assumption that
most tumorigenic mutations only slightly tilt the
survival odds in the mutant stem cell’s favor [6,7], it
follows that oncogenic mutations occur much more fre-
quently than we measure experimentally, because the
mutant is randomly lost from the stem cell niche before
it can become dominant and persist in the population.
Because we observed neutral drift in both normal (APC
wild type) and mutant (APC+/- and APC-/-) crypts, this
same argument applies equally both in the pre-neoplastic
phase and in established adenomas. This has important
implications for chemoprevention of cancer: since most
potentially carcinogenic mutants will naturally go extinct,
it might not be necessary to design drugs specifically to
kill the mutants. Instead, tipping the balance of evolution
within the crypt to make it more difficult for any stem cell
clonal replacement rate, but importantly both APC+/- and
APC-/- stem cell niches remain governed by neutral
drift dynamics.

Crypt fission is slow, even in adenomas

After a mutant crypt has acquired a carcinogenic muta-
tion, to spread further in the colonic mucosa, the
mutant crypt must then undergo successive ‘fission’
events. Colorectal neoplasia is thus a product of aber-
rant crypt fission, rather than aberrant cell growth
per se, and understanding the drivers of crypt fission is
of paramount importance.

Crypt fission events can be detected using the CCO
lineage labeling technique described above. A patch of
two or more adjacent crypts bearing the same mutation
implies they have arisen from the ancestral crypt via a
fission event, therefore analyzing the frequency and
number of these clonal patches of mutant crypts can pro-
vide insight into the frequency of crypt fission. Using
mathematical modeling, we inferred that fission occurs
at a very low rate in the normal colon, with crypts
dividing only once or twice in a lifetime, although
there is substantial variation between individuals [5].
This rate is not altered significantly in the FAP APC+/-
colon, whereas we measured that APC-/- adenomatous
crypts in small adenomas had a tenfold increased rate
of crypt fission. Although substantial, this increase is
still quite small. The apparent relatively small effect of
APC mutation on fission rates is likely to be because our
study only involved small adenomas – and we suppose
that crypt fission is further accelerated in large adeno-
amas and carcinomas because of additional mutations.
However, a slow growth rate in adenomas is consistent
with observations in the clinic [9,10], and time to pro-
gression calculated by comparative lesion sequen-

Nevertheless, and somewhat reassuringly, the implica-
tion of a slow adenoma growth rate is that there is a long
window of opportunity to detect such lesions during
screening. This does, indeed, seem to be the case, with
four adenomas for every cancer detected in the UK’s
Bowel Cancer Screening Programme [12]. Given the slow
evolution of an adenoma to carcinoma, the challenge
now is to find a robust way to determine which of the screen-detected adenomas have potential to progress to cancer within the lifetime of the patient, and to understand the timescale of this transformative process, in order to optimize screening programs, specifically to rationally choose the optimal screening interval.

**Summary**

The formation of a CRC is an evolutionary process, and our recent work has provided quantitative measurement of stem cell and crypt evolution in the human colon. This detailed mechanistic understanding is important, because only by understanding exactly how the process works can we design optimal strategies to intervene in the process and prevent cancer. We have shown that stem cell evolution in the colon crypt is governed by rampant competition between stem cells and by slightly tweaking this competition – to make it harder for any cell to ‘win’ – we might be able to prevent cancer-causing mutations persisting in the crypt. Our measurement of the slow growth of adenomas can be built upon to mechanistically justify bowel cancer screening intervals.

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