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Evolutionary Stem Cell Poker and Cancer Risks: The Paradox of The Large And Small Intestines

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Abstract

Purpose of review: Recent studies demonstrate that normal human tissues accumulate substantial numbers of somatic mutations with aging, to levels comparable to their corresponding cancers. If mutations cause cancer, how do tissues avoid cancer when mutations are unavoidable?

Recent findings: The small intestines (SI) and colon accumulate similar numbers of replication errors, but SI adenocarcinoma is much rarer than colorectal cancer. Both the small and large intestines are subdivided into millions of small neighborhoods (crypts) that are maintained by small numbers of stem cells. To explain the SI cancer paradox, four fundamental evolution parameters (mutation, drift, selection, and population size) are translated to crypts.

Summary: The accumulations of driver mutations in a single stem cell may be analogous to an evolutionary poker game. The rarity of SI cancer may reflect that SI crypts are smaller and have fewer stem cells than the colon, which reduces the numbers of cells at risk for mutation and perhaps selection efficiency. Tissue microarchitecture may physically modulate cancer evolution by controlling the numbers of directly competing neighboring cells. A better understanding of the SI cancer paradox may illuminate how tissues naturally avoid cancers when mutations are unavoidable.

Keywords

stem cell niche; somatic mutation; replication errors; neutral evolution; small intestinal adenocarcinoma; selection efficiency

Introduction

Tumor progression is a multistep evolutionary process where somatic alterations that accumulate stepwise in a single cell eventually lead to neoplasia. Although progression is typically illustrated with visible changes in tumor phenotypes and sizes, recent advances have documented that many somatic mutations accumulate with age in normal appearing human tissues with numbers often comparable to the numbers of mutations found in

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corresponding tumors from the same tissues. Examples include the skin, liver, brain, blood, and the small and large intestines (1–5). These new data provide opportunities to explore progression before visible tumorigenesis. What sort of evolutionary framework should be used to describe evolution without discernable phenotypic changes? This question is applied to the intestines, specifically to the paradox that in humans, cancers are common in the large intestines but very rare in the small intestines (SI).

Two General Types of Evolution

Darwinian evolution is commonly applied to tumor progression, as exemplified by the adenoma-cancer sequence. Mutations accumulate in a population of cells and evolution “occurs” when one cell acquires a fitness advantage and that clone expands. The only way for a mutation to confer a fitness (survival and/or reproductive) advantage, is to change the phenotype of the cell. Hence, Darwinian evolution encompasses both changes in genotype and phenotype. Darwinian evolution is hard to observe in normal tissues, because by definition, visible evolution does not “occur”. Evolution without measurable phenotypic changes is “non-Darwinian” and is characterized as “neutral evolution” (6). The neutral theory of molecular evolution holds that at the molecular level most evolutionary changes and most of the variation within and between species is not caused by natural selection but by the drift of mutant alleles that are neutral - that is, they have no effect on the survival or reproduction of the organism.

Darwinian and neutral evolution can together describe the lifelong process of progression from the zygote to a visible tumor. Neutral evolution better describes the accumulation of genomic variation that occurs between visible Darwinian phenotypic changes. Neutral evolution is important because it modulates the genomic variation eventually required for Darwinian evolution. The longest progression interval for most tumors is the neutral evolution between the zygote and the first visible tumor.

Small Intestinal Cancer Is Rare: A Mutation Paradox

The human SI is longer than the colon (~30 feet versus ~6 feet), but colorectal cancers (CRCs) are ~44 times more common (7). Driver mutations are similar between SI adenocarcinomas and CRCs although APC mutations are less common (7). From a Darwinian perspective, typically “nothing” happens in the SI. However, new sequencing data (1) indicate that measurable numbers of mutations accumulate in the SI and colon.

Fewer SI cancers could be explained if mutations were much less common in normal SI. However, somatic mutations are just as common in the colon and SI (Table 1). The difference could also be because only a small fraction of cancer mutations accumulate in normal intestines, but normal intestines accumulate almost as many mutations as CRCs. Finally, mutation mechanisms may differ between the SI and the colon, especially because the colon has a rich microbiome and the SI is virtually sterile. However, mutation spectra are similar between the SI and colon (1) and both are consistent with aging or replication errors. Both the colon and SI are highly mitotic tissues with mitotic stem cells (8), and the age-related accumulation of large numbers of intestinal somatic mutations may reflect that active cell division is needed for normal tissue hemostasis.

In summary, although SI cancers are rare, the same numbers and types of mutations accumulate with aging in the colon and SI. One cannot distinguish the SI and colon by their mutations. Indeed, one cannot easily distinguish normal intestines from age-matched CRCs by the numbers of mutations. If mutations cause cancer, how does the SI avoid the cancer prone fate of the colon?

Crypts and Their Four Fundamental Evolution Parameters

Evolution has four major related parameters —mutation, drift, selection, and population size. Population size is of paramount importance because only interacting neighboring cells compete for survival. For the intestines, the evolutionary unit or neighborhood is the crypt. Both the SI and colon are subdivided into millions of small neighborhoods or crypts (Fig 1A). Notably, SI crypts are smaller than colon crypts (respectively ~400 versus ~2000 cells per crypt). Mutations can be measured by sequencing, but drift and selection are more nebulous parameters because they depend on the surrounding environment. Drift acts on neutral or passenger mutations, and driver mutations confer selection.

A crypt has a stem cell hierarchy with small numbers of stem cells at the base and more numerous differentiated cells that migrate upwards and die within several days (Fig 1A). This crypt architecture physically reduces cancer risks because mutations that arise in the more numerous differentiated cells are lost, and even mutations that occur in stem cells can be limited to only spread within individual crypts (9). Multiple mutations can only accumulate in long-lived stem cell lineages. Intestinal stem cells are mitotic and divide about once a day in mice (8). Stem cells are maintained by a niche mechanism where the numbers of stem cells are constant but individual stem cell lineages may either expand or become extinct. This stem cell turnover is random and eventually one stem cell lineage populates the entire crypt through neutral drift (10, 11). Crypt niche succession by a single stem cell lineage takes several months in mice (10, 11) and perhaps eight years in human colon crypts (12).

Random crypt stem cell survival exemplifies neutral evolution (Fig 1A) because the population size is constant and there are no visible changes in phenotype, but individual stem cell lineages undergo expansion or extinction. This ongoing neutral evolution is independent of mutations and can be measured with visible neutral fate markers such as beta-galactosidase (8). Neutral mutations that arise as replication error are “passengers” that depend on the survival of their stem cells. Because there are multiple stem cells per crypt, the fate of each new mutation is initially uncertain. The fates of most replication errors are extinction because only one stem cell lineage survives neutral drift. With an effective population size of “N” numbers of stem cells per crypt, the probability of survival is $1/N$. However, when the passenger mutation occurs in the stem cell that undergoes niche succession, it is forever “fixed” because it is now present in all the cells of its crypt.

The Crypt is a Crucible

With multiple niche stem cells, every new mutation is initially “judged” and either fixed or discarded. This judgement is random with passenger mutations, but a new driver mutation in a single stem cell should achieve niche succession and fixation by displacing surrounding

less fit stem cells. Interestingly, mouse studies illustrate that even strong driver mutations in single stem cells are often lost (13). A single normal stem cell drifts to fixation ~20% of the time, and a single stem cell with a Kras (G12D) mutation surrounded by normal stem cells becomes fixed ~75% of time. A stem cell with a single Apc allele is fixed about one third of the time but is lost about two-thirds of the time. A single stem cell with homozygous Apc loss (–/–) is fixed only slightly more than half of the time. A single stem cell with a Tp53 mutation is fixed no more frequently than a normal stem cell and only shows a selective niche survival advantage in the setting of inflammation.

These mouse studies show that selection is imperfect even for strong driver mutations. The loss of driver mutations from crypts is a potentially potent anti-cancer mechanism. Given that random replication errors should produce similar numbers of driver mutations in the SI and colon, the differences in cancer frequencies could be explained if SI crypts were less efficient at accumulating drivers. Interestingly, Fisher-Wright population genetics predicts that selection becomes increasingly less efficient as population sizes decrease because stochastic survival (drift) becomes more important (14).

Crypt stem cell population sizes are extremely small, with estimates of less than 20 per mouse crypt (15). In such very small populations, evolution has a harder time distinguishing between passengers and drivers (14). The extreme case is a crypt with a single mitotic stem cell lineage, where there is neither drift nor selection, and fixation is the same for passenger and driver mutations. Here the accumulation of multiple driver mutations in a single stem cell is essentially pure luck. When there are multiple stem cells per crypt, both skill (selection) and luck (drift) can modulate mutation accumulation.

How Crypts Might Play Evolutionary Stem Cell Poker: Luck versus Skill

Poker, a game of skill and chance, can illustrate evolutionary principles and help explain how the SI and colon can accumulate different mutation combinations even though they both draw from the same deck at the same rates (i.e. accumulate the same numbers of replication errors). The four crypt evolution parameters are analogous to the rules of an imaginary poker game (Fig 1B) where the goal is to accumulate a very rare hand (“royal flush”). There are five rounds of play and variable numbers of cards (“niche size”) can be drawn each round, but only one card (or mutation) is retained after each round. There are four types of players (or crypts). The first player (“Dumb Luck”) simply draws a single card each round and ends up with five cards. The second player (“No Brains”) draws four cards each round, randomly keeps one, and also ends up with five cards. The third player (“The Novice”) also draws four cards each round and tries to select the better card. The final player (“Card Shark”) draws six cards each round and inevitably selects the best card.

In this crypt evolutionary poker game, the cards are random mutations, the numbers of cards drawn each round are the numbers of stem cells per crypt, and which card is kept each round depends on the skill of the player to select the best card. Mouse crypts play like “The Novice” because they have multiple niche stem cells and can select driver mutations, even though they often discard strong driver mutations (12). In this game, “winning” is still possible without any skill (“Dumb Luck” and “No Brains”), but is more likely when drawing more cards and having the skill to select better cards (“The Novice” and “Card Shark”).

Both the degree of skill (selection efficiency) and stem cell numbers per crypt are currently unknown for human intestines, but potentially one answer to the SI cancer paradox is that SI crypts have fewer stem cells than colon crypts (Fig 1A), and/or selection is weaker in SI crypts. As noted above, Fisher-Wright population theory indicates that N may inherently modulate the relative strengths of drift and selection because selection is weaker in very small populations.

Unraveling Evolutionary Poker

In the “Tournament of Cancer”, the odds of cancer may reflect that tissues play stem cell evolutionary poker differently. Sequence data measure “final hands” but tell little about how crypts accumulate their mutations. Do the numbers of stem cells per crypt differ between the SI and colon? Measuring the numbers of stem cells per crypt is difficult because the experimental fate marker approaches used in mice (8) are impractical. However, other approaches that measure somatic alterations indicate that human crypts also contain multiple stem cells (12,16–20). A recent study indicated that certain selective mutations can increase colon crypt fission (from one to two crypts) above a baseline rate of ~0.7% per year, which can further favor progression (20). Very few studies have compared human SI and colon crypts, and a study with passenger methylation indicated that human SI and colon crypts contain multiple mitotic stem cells that divide at similar rates (21). Deep sequencing can potentially count niche stem cells because a crypt with more niche stem cells would have more subclonal mutations. Comparisons of synonymous and nonsynonymous mutations can detect somatic cell selection (22) and stochastic drift will tend to accidentally fix many more deleterious (but non-lethal) mutations. If selection is less efficient in the SI, more deleterious mutations should be found in normal SI compared to normal colon. Random replication errors and inefficient selection that fixes more deleterious than advantageous mutations may help explain why our intestines (and other tissues) unfortunately become less fit instead of more fit with aging.

Summary: Mutations Without Cancer

The intestinal crypt microarchitecture may represent an evolutionary compromise that balances the relative morbidity of infections and cancer. Multiple stem cells per crypt ensure that the damage and loss of any single stem does not result in loss of the crypt. Small stem cell numbers limit the numbers of cells at risk and may fortuitously decrease the efficiency of selection, making it more difficult to accumulate multiple drivers in a single cell.

The much greater incidence of CRC may reflect that larger colon crypts have more stem cells because the microbiome exposed colon is more prone to damage. This mechanism is structural (Fig 1A) and hence could help inherently explain the paradox of why SI cancer is so reliably uncommon even though mutations uniformly accumulate in the intestines. Like the poker game (Fig 1B), luck is still paramount when only very rare mutation combinations result in cancer. Greater numbers of colon crypt stem cells may change the odds by increasing the numbers of cells at risk and the efficiency of selection. Along these lines, many behavioral modifications alter CRC risks, but their underlying prevention mechanisms are uncertain, especially because the intestine remains normal appearing. However, because stem cells are morphologically indistinguishable from their neighbors, the numbers of crypt

stem cells may be surreptitiously altered to either increase or decrease the odds of cancer though alterations in WNT signaling (23). Even minor changes in the numbers of cards (stem cells) drawn during each round of evolutionary poker could measurably alter the odds. Indeed, why specific driver mutations such as in APC may be so common in CRC may be because they potentially increase the numbers of niche stem cells, further increasing the odds of progression to cancer. Differences between the SI and colon may hold the key on how to naturally prevent cancer when mutations are unavoidable.

Acknowledgments:

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References

Recently published papers of particular interest have been highlighted as:

- Of importance
- Of major importance

1**^{*}. Blokzijl F, de Ligt J, Jager M, et al. (2016) Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* 538:260–264.

This paper documents with organoid cultures and genome sequencing the age-related increases in somatic mutations in the normal human SI, colon and liver.

[PubMed: 27698416]

2**^{*}. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, Wedge DC, Fullam A, Alexandrov LB, Tubio JM, Stebbings L (2015) High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348:880–6.

This paper documents the high mutation burdens in normal human skin with multiple small skin regions and sequencing.

[PubMed: 25999502]

3. Lodato MA, Rodin RE, Bohrsen CL, Coulter ME, Barton AR, Kwon M, Sherman MA, Vitzthum CM, Luquette LJ, Yandava CN, Yang P, Chittenden TW, Hatem NE, Ryu SC, Woodworth MB, Park PJ, Walsh CA (2018) Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science* 359:555–559 [PubMed: 29217584]

4. Genovese G, Kähler AK, Handsaker RE, et al. (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 371:2477–87 [PubMed: 25426838]

5. Jaiswal S, Fontanillas P, Flannick J, et al. (2014) Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 371:2488–98 [PubMed: 25426837]

6. Duret L (2008) Neutral theory: The null hypothesis of molecular evolution. *Nature Education* 1:218

7. Raghav K, Overman MJ Small bowel adenocarcinomas—existing evidence and evolving paradigms (2013) *Nat Rev Clin Oncol* 10:534–544 [PubMed: 23897080]

8. Barker N, et al. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449:1003–1007 [PubMed: 17934449]

9. Cairns J (1975) Mutation selection and the natural history of cancer. *Nature* 255:197–200. [PubMed: 1143315]

10. Lopez-Garcia C, Klein AM, Simons BD, Winton DJ (2010) Intestinal stem cell replacement follows a pattern of neutral drift. *Science* 330:822–825 [PubMed: 20929733]

11. Snippert HJ, et al. (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143:134–144 [PubMed: 20887898]

10. Yatabe Y, Tavaré S, Shibata D (2001) Investigating stem cells in human colon by using methylation patterns. *Proc Natl Acad Sci U S A* 98:10839–44
- 13**. Vermeulen L, et al. (2013) Defining stem cell dynamics in models of intestinal tumor initiation. *Science* 342:995–998
- Demonstrates that crypt driver mutation fixation is probabilistic rather than deterministic.

[PubMed: 24264992]

14. Whitlock MC (2000) Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution; international journal of organic evolution* 54:1855–1861
15. Clevers H (2013) The intestinal crypt, a prototype stem cell compartment. *Cell* 154:274–84 [PubMed: 23870119]
16. Campbell F, Williams GT, Appleton MA, Dixon MF, Harris M, Williams ED (1996) Postirradiation somatic mutation and clonal stabilisation time in the human colon. *Gut* 39:569–573 [PubMed: 8944567]
17. Greaves LC, Preston SL, Tadrous PJ, Taylor RW, Barron MJ, Oukrif D, Leedham SJ, Deheragoda M, Sasieni P, Novelli MR, Jankowski JA, Turnbull DM, Wright NA, McDonald SA (2006) Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. *Proc Natl Acad Sci U S A* 103:714–719
18. Baker AM, Cereser B, Melton S, Fletcher AG, Rodriguez-Justo M, Tadrous PJ, Humphries A, Elia G, McDonald SA, Wright NA, Simons BD, Jansen M, Graham TA (2014) Quantification of crypt and stem cell evolution in the normal and neoplastic human colon. *Cell Rep* 8:940–7 [PubMed: 25127143]
19. Kang H, Shibata D (2013) Direct measurements of human colon crypt stem cell niche genetic fidelity: the role of chance in non-Darwinian mutation selection. *Front Oncol* 3:264 [PubMed: 24133655]
- 20**. Nicholson AM, Olpe C, Hoyle A, Thorsen AS, Rus T, Colombé M, Brunton-Sim R, Kemp R, Marks K, Quirke P, Malhotra S, Ten Hoopen R, Ibrahim A, Lindskog C, Myers MB, Parsons B, Tavaré S, Wilkinson M, Morrissey E, Winton DJ (2018) Fixation and spread of somatic mutations in adult human colonic epithelium. *Cell Stem Cell* 22:909–918
- Infers human colon crypt stem cell dynamics and crypt fission rates with multiple somatic fate markers.

[PubMed: 29779891]

21. Kim JY, Siegmund KD, Tavaré S, Shibata D (2005) Age-related human small intestine methylation: evidence for stem cell niches. *BMC Med* 3:10 [PubMed: 15975143]
- 22**. Martincorena I, Raine KM, Gerstung M, Dawson KJ, Haase K, Van Loo P, Davies H, Stratton MR, Campbell PJ (2017) Universal patterns of selection in cancer and somatic tissues. *Cell* 171:1029–1041
- Illustrates how somatic cell selection can be inferred from somatic mutations.

[PubMed: 29056346]

23. Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 346:1248012 [PubMed: 25278615]

Fig 1. Evolutionary poker played in intestinal crypts

A) The intestines are subdivided into millions of crypts that are maintained by a stem cell hierarchy. Niche stem cells at crypt bases are mitotic and divide to yield either one stem and one differentiated daughter (renewal), two stem cell daughters (expansion), or two differentiated daughters (extinction). Eventually all present-day stem cell lineages are lost except one by a process called neutral drift. Therefore, each new mutation can either be lost (the usual fate) or fixed and become present in all crypt cells if it occurs in the stem cell that

attains niche succession. SI crypts are smaller than colon crypts and may have fewer niche stem cells.

B) The process of discarding and fixing random replication errors within crypts is analogous to an evolutionary poker game where multiple cards can be drawn but only one card is retained during each round of play. By analogy, cards are mutations, the numbers of drawn cards are the numbers of niche stem cells, and the ability to pick the better card reflects the balance between selection and drift (see the text). Any strategy can “win”, but the odds are greater if one draws more cards each round and keeps the better card. The size differences between SI and colon crypts may physically explain why SI cancers are rarer than CRCs even though they both accumulate the same numbers of mutations (cards) in their final “hands”.

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Table 1:

Human Small and Large Intestines

Feature	SI	Colon	CRC (non-mutator)
Mutations at age 70	~0.8	~0.8	~1–5 per Mb
Mutation spectra	“Aging”	“Aging”	“Aging”
Crypt size	~400 cells	~2,000 cells	
Length	~30 feet long	~6 feet long	
Cancer incidence	very low	very common	