Unwanted Evolution

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CANCER

Unwanted Evolution

The dynamics of stem cell replacement at the bottom of the intestinal crypt is visualized and quantified.

Ivana Bozic[1,2] and Martin A. Nowak[1,2,3]

We mostly think of evolution as a process that has the power to build structures of incredible beauty and functionality, like multicellular organisms, the nervous system, and human language. But evolutionary dynamics can also lead to processes that are not wanted, such as cancer, because they oppose the survival interests of the organism. The somatic evolution of cancer is a consequence of our cells being individual replicators. Upon acquiring mutations, cells can revert to their primitive program of proliferation, competition for survival, and selection of the fittest. On page 995 of this issue, Vermeulen *et al*. (*1*) demonstrate how multicellular organisms keep this unwanted evolution in check. By quantifying the effects of mutations that frequently occur during colorectal tumor development, the authors show that intestinal tissue architecture acts as a suppressor of selection.

One way to prevent unwanted evolution is to hold somatic mutation rates low. The mutation rate in healthy human cells is on the order of 10^{-10} to 10^{-9} per nucleotide base pair per cell division (*2*), perhaps as low as possible given reasonable energy constraints. What about selection—can it be suppressed? There are population structures where this is the case, such as when a large population of differentiated cells is continuously renewed by the asymmetric division of stem cells (*3*, *4*). It has been hypothesized that many epithelial tissues, which are especially susceptible to cancer because of their many cell divisions, are designed to suppress selection (*3*). Intestinal epithelium is replenished by equipotent stem cells residing at the bottom of crypts. These stem cells are continuously replacing each other in a random fashion (*5*, *6*). Most intestinal tumors are thought to initiate from a transformed (mutated) stem cell, because genomic alterations occurring in differentiated cells would be "flushed out" as a result of the specific tissue architecture (differentiated cells are continuously pushed upward in the crypt and eventually undergo programmed cell death at the top of the crypt) (*3*, *7*). The initiating genetic event in the majority of colorectal cancers is an inactivation of the *APC* tumor suppressor gene, often followed by mutations in the oncogene *KRAS* and the tumor suppressor *P53*, among other genes (*8*).

To measure the effect of initial genetic changes occurring in colorectal cancer, Vermeulen *et al*. visualized and quantified the dynamics of stem cell replacement in the mouse intestine when one of the stem cells in the crypt contains a particular change, such as an inactivation of one or both copies of *Apc*, activation of *Kras*, or a mutation in *p53*. The authors demonstrate that the evolutionary dynamics of stem cells can be well described by a simple mathematical model that assumes a ring of five to seven stem cells at the base of the crypt. A mutant stem cell replaces its neighboring wild-type stem cell with probability P_R, whereas the wild-type cell replaces the mutant with probability $1 - P_R$ (see the figure).

Vermeulen *et al*. show that *Apc* inactivation and *Kras* activation increase the probability that a stem cell carrying those alterations replaces a neighboring wild-type stem cell. It is generally believed that inactivation of one of the *Apc* alleles does not lead to a phenotypic change, but the authors found that *Apc* exhibits some degree of haploinsufficiency (*9*): Even a single inactivated *Apc* copy ($Apc^{+/-}$) leads to some selective advantage over wild-type stem cells. The probability that an $Apc^{+/-}$ mutant cell replaces an $Apc^{+/+}$ wild-type cell is estimated to be $P_R = 0.62$. Stem cells with both copies of *Apc* inactivated, $Apc^{-/-}$, replaced neighboring $Apc^{+/-}$ cells with probability $P_R = 0.69$. Activation of *Kras* had

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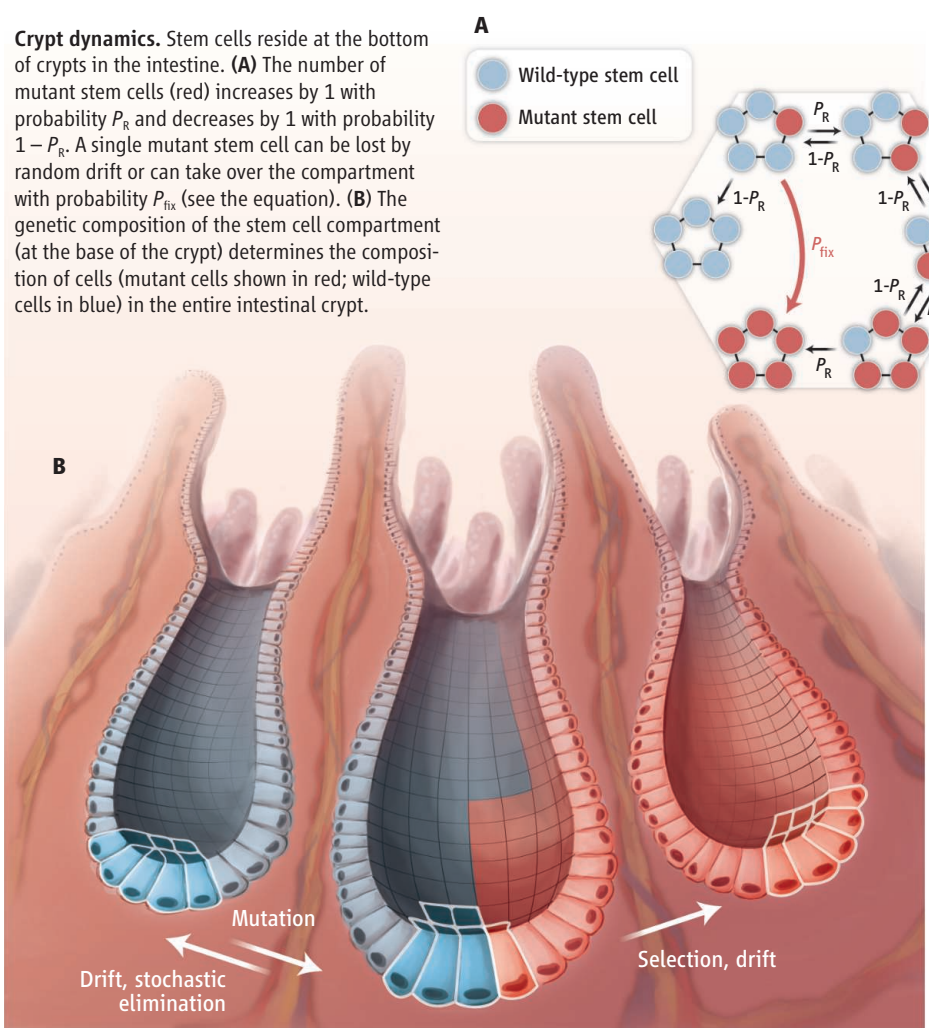

Crypt dynamics. Stem cells reside at the bottom of crypts in the intestine. **(A)** The number of mutant stem cells (red) increases by 1 with probability P_R and decreases by 1 with probability $1 - P_R$. A single mutant stem cell can be lost by random drift or can take over the compartment with probability P_{fix} (see the equation). **(B)** The genetic composition of the stem cell compartment (at the base of the crypt) determines the composition of cells (mutant cells shown in red; wild-type cells in blue) in the entire intestinal crypt.

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the most pronounced effect, with a 78% chance of replacing an adjacent wild-type stem cell. The authors also found that *p53* mutations provide a selective advantage, but only in gut affected by inflammation (P_R = 0.58) and not in normal conditions (P_R = 0.48).

Although mutant stem cells are more likely to replace wild-type stem cells than vice versa, they can nevertheless be lost by random drift (when the frequency of mutant cell in a population decreases until it disappears). The probability that a single mutant stem cell reaches fixation in a crypt (takes over the entire stem cell compartment of the crypt) can be calculated as

$$P_{\text{fix}} = \frac{1 - \left(\frac{1 - P_R}{P_R}\right)}{1 - \left(\frac{1 - P_R}{P_R}\right)^N}$$

in which N is the number of stem cells per crypt. Assuming $N = 5$ stem cells in a crypt and using the experimentally derived values for P_R, the probability for a single $Apc^{+/-}$ stem cell to take over the crypt is 0.42. Similarly, the probability that a single $Apc^{-/-}$ cell reaches fixation in an $Apc^{+/-}$ background is 0.56. The authors report similar fixation probabilities based on direct measurement.

This detailed quantitative information about the initial steps in the somatic evolution of a solid cancer makes an important connection between theory and experiment. The values for the selective advantage of frequently occurring mutations can provide precise guidelines for future studies of tumor initiation and progression (*10*–*15*). As Vermeulen *et al*. point out, a similar approach can be used to investigate the role of chromosomal instability in tumor initiation. Their findings also raise new questions about the events that follow the fixation of a mutation in a single crypt, including quantification of a field cancerization effect and the change in dynamics and tissue morphology caused by the subsequent mutations. Most important, this exciting study opens the door for a quantitative understanding of cancer initiation. One can also envisage the possibility of using the devised experimental framework for the development and testing of drugs that attack mutated stem cells, thereby preventing the process of cancer initiation.

References

1. L. Vermeulen *et al*., *Science* **342**, 995 (2013).
2. D. J. Araten *et al*., *Cancer Res.* **65**, 8111 (2005).
3. M. A. Nowak, F. Michor, Y. Iwasa, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14966 (2003).
4. E. Lieberman *et al*., *Nature* **433**, 312 (2005).
5. C. Lopez-Garcia, A. M. Klein, B. D. Simons, D. J. Winton, *Science* **330**, 822 (2010).
6. H. J. Snippert *et al*., *Cell* **143**, 134 (2010).
7. N. Barker *et al*., *Nature* **457**, 608 (2009).
8. S. Jones *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4283 (2008).
9. N. L. Solimini *et al*., *Science* **337**, 104 (2012).
10. R. Meza, J. Jeon, S. H. Moolgavkar, E. G. Luebeck, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16284 (2008).
11. I. Bozic *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18545 (2010).
12. R. Durrett *et al*., *Theor. Popul. Biol.* **78**, 54 (2010).
13. C. Tomasetti, B. Vogelstein, G. Parmigiani, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 1999 (2013).
14. B. Vogelstein *et al*., *Science* **339**, 1546 (2013).
15. D. Wodarz, N. L. Komarova, *Computational Biology of Cancer: Lecture Notes and Mathematical Modeling* (World Scientific, Hackensack, NJ, 2008).

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MATERIALS SCIENCE

Metamaterials Beyond Optics

The area of metamaterials may expand to find application in mechanics, acoustics, and thermodynamics.

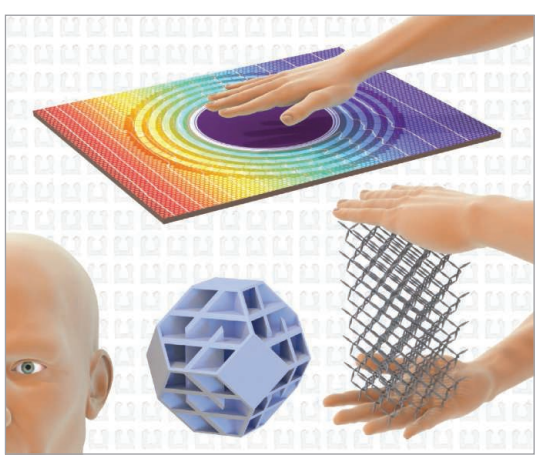
Martin Wegener

So far, the field of metamaterials has largely dealt with negative refractive indices in optics and invisibility cloaks (*1*–*3*). However, the underlying idea of designing material properties is not that narrow. More broadly, one rationally designs a subwavelength unit cell from existing constituent materials and (periodically) arranges it into an artificial solid. The properties of that solid are then determined by structure rather than chemistry and can be tailored, extreme, or even qualitatively unprecedented. Rational design is the key and makes metamaterials a rather particular class of composite materials.

In terms of application, some aspects of metamaterials such as negative phase velocities of light (*1*, *2*), invisibility cloaking (*4*, *5*), or unusual optical nonlinearities (*6*) are fascinating but are not likely to soon appear in products, because optical absorption (loss) is too high and in part fundamentally unavoidable. Moreover, the inexpensive manufacturing of complex large-volume three-dimensional optical metamaterials is still a formidable challenge in itself (*2*).

Why, then, don't we go beyond optics and also consider other material aspects (*7*) such as thermal, acoustic, elastic, or irreversible nonlinear mechanical properties? The corresponding wavelengths and length scales range from tens of micrometers to centimeters, rather than nanometers in optics. Hence, fabrication limitations are relaxed, thus easing real-world applications. Moreover, one lesson learned from electromagnetism is that off-resonant constituents enable low losses. In the visible spectrum, however, this results in a contrast in refractive index of no more than 3 for available nonabsorbing dielectrics. Some theoretical blueprints demand constituent-material contrasts in the range of tens or hundreds. Such values are accessible in mechanics and thermodynamics (see the figure).

Consider simple mechanical waves in elastic solids. They exhibit three orthogonal polarizations, one longitudinal (like sound waves in air or water) and two transverse (like electromagnetic waves). This complex



Seeing, hearing, feeling. While optics has been in the foreground of metamaterials research, opportunities arise in other areas such as acoustics, mechanics, and thermodynamics (heat conduction and diffusion). In all of these, larger lattice constants ease the fabrication requirements and losses can be much lower or absent.

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