Cell

Cancer Evolution: No Room for Negative Selection

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https://doi.org/10.1016/j.cell.2017.10.039

In this issue of *Cell*, Martincorena et al. and Campbell et al. interrogated the selection dynamics during tumor evolution using large-scale genomics datasets. They found that somatic mutations in cancer are largely neutral, highlighting a near-complete absence of negative selection. Neutral evolution enables tolerance of hypermutation, which defines a surprisingly large fraction of adult cancer.

Cancer genomics has transformed our understanding of the genetic causes of tumorigenesis (Garraway and Lander, 2013), enabling unbiased discovery of oncogenes and tumor suppressors. From its inception, the cancer genomics field implicitly relied on the assumption that causative somatic mutations undergo positive selection—and will therefore recur across tumors—in large patient cohorts (Lawrence et al., 2014). As the size of cancer datasets dramatically increases, we now begin to engage more explicitly with the evolutionary principles that underlie cancer and dissect the forces that shape the malignant genome.

In this issue of *Cell*, Martincorena et al. (2017) applied dN/dS, a tool commonly used in the study of organismal evolution, to the Tumor Cancer Genome Atlas (TCGA) in order to identify selection pressures exerted on mutant somatic cancer alleles. This method utilizes synonymous mutations (single-nucleotide substitutions that result in no change in the amino acid composition) as the evolutionarily neutral mutational background. A comparison of the density and frequency of non-synonymous mutations to this background provides a quantitative measure of whether such mutations are subject to positive, negative, or no selection. For instance, in the event that a non-synonymous mutation is positively selected, the ratio of the non-synonymous-to-synonymous mutations (dN/dS) will be >1. On the other hand, negative selection, or the absence thereof, would yield dN/dS ratios smaller than or equal to 1, respectively (Figure 1A). To apply this tool to the cancer evolution context, the authors made a number of important refinements to account for recently described key mutation-rate modifiers such as transcribed strand bias, cancer-specific mutational signatures, and variations in chromatin structure.

Applying the analysis to a large dataset comprising 7,664 tumors across 29 cancer types, the authors confirmed that the vast majority of non-synonymous mutations are not subject to selection at all and that only a minority of mutations (~5%) are positively selected, resulting in dN/dS values that are minimally >1 (Figure 1B). This result conforms to the general consensus that the limited number of driver mutations is vastly outnumbered by passenger mutations. Indeed, the dN/dS-based identification of driver cancer genes and the estimated number of driver mutations per cancer are largely in agreement with previous studies (Lawrence et al., 2014; Tomasetti et al., 2015).

A striking finding of this analysis is the near-complete absence of negative or purifying selection. This stands in stark contrast with the dN/dS analysis of the germline human genome, which at a ratio of 0.08, shows the fingerprints of powerful negative selection. Why are non-synonymous mutations so effectively weeded out of the germline genome but overwhelmingly tolerated in the somatic genome? This may reflect fundamental differences in the two evolutionary processes. Cancer evolution is remarkably short in comparison to organismal evolution, limiting the effectiveness of negative selection. Moreover, tumor cells reproduce asexually without chromosome recombination, which facilitates the purging of deleterious alleles. Given the lack of recombination, the accumulation of potentially deleterious mutations may be the consequence of their co-inheritance with alterations that confer a significant fitness advantage, a phenomenon referred to as Muller's Ratchet (Haigh, 1978; Figure 1C).

Nonetheless, the paucity of purifying selection in cancer suggests that the human genome, which has evolved within the constraints of multicellularity, is highly resilient to alterations at the somatic level—when cells rescind their multicellular contract in favor of unicellular growth. Furthermore, cancer resilience might be reinforced through aneuploidy and whole-genome doubling. These copy number abnormalities, observed in two-thirds of human cancer, enable tumor cells to buffer deleterious non-synonymous mutations, through the presence of multiple copies of many genes (Figure 1C). Indeed, recent work in yeast has demonstrated that even gene essentiality can be bypassed through aneuploidy (Liu et al., 2015).

The absence of negative selection in cancer may explain tolerance for an increased mutational burden. It has long been known that a small subset of tumors exhibit a hypermutation phenotype linked to microsatellite instability and defects in DNA polymerases. Here, Campbell et al. (2017) apply targeted gene panel sequencing to more than 81,000 tumors and make the important discovery that hypermutation (>10 somatic mutations

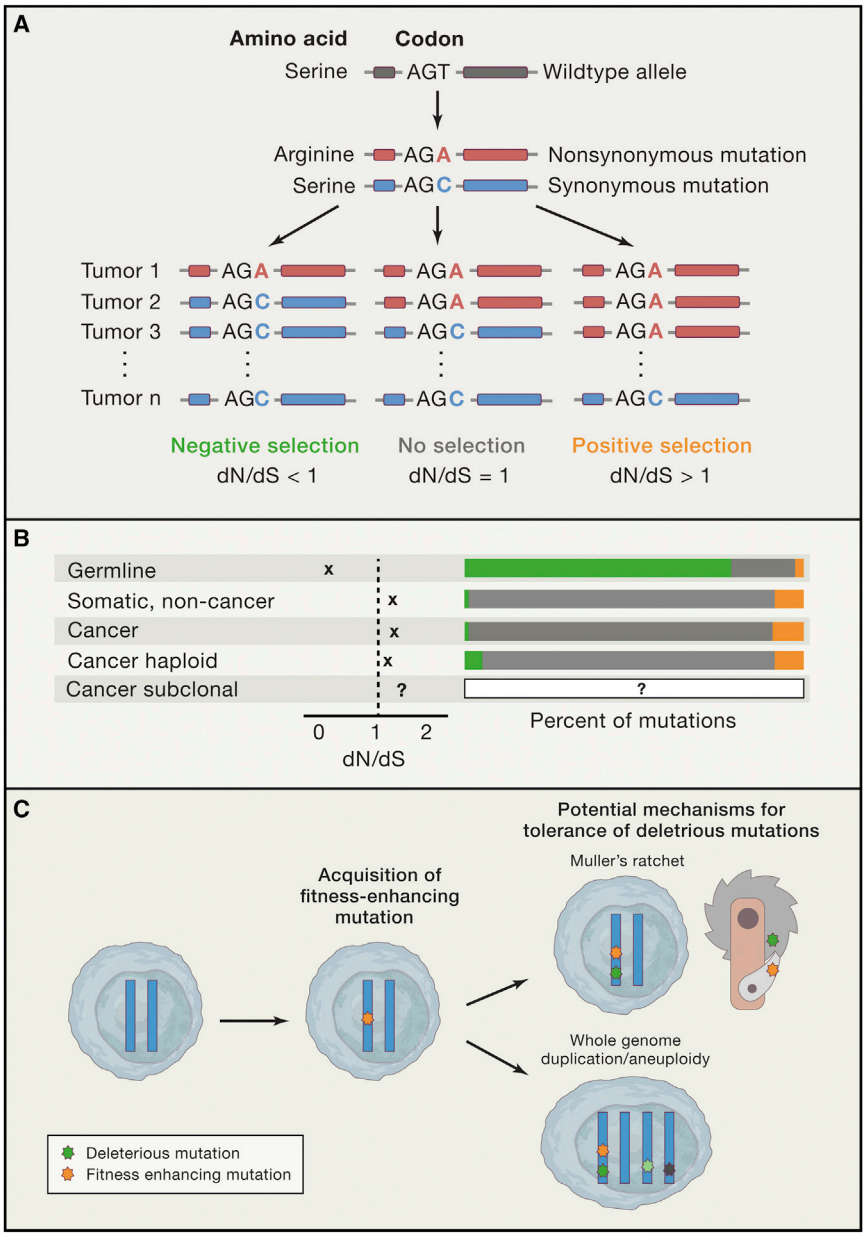

Figure 1. Selection Dynamics in Cancer Evolution

(A) The ratio of non-synonymous-to-synonymous mutations in any given gene (dN/dS) can be used to identify selection pressures acting on mutations in the cancer genome.

(B) Martincorena et al. identify that unlike germline evolution, where most mutations are subject to negative "purifying" selection, cancer mutations arise in an evolutionary neutral framework with a small bias for positive selection.

(C) A number of mechanisms can enable tolerance for deleterious mutations during tumor evolution in cancer. Two such mechanisms are the co-inheritance of deleterious mutations with fitness-enhancing mutations, locked in due to the lack of recombination (top), and whole-genome doubling or aneuploidy that may help buffer deleterious mutations through multiple gene copies (bottom).

per megabase) is more prevalent than previously appreciated, affecting nearly 17% of adult cancers. Hypermutation was also detected in tumor types not commonly associated with increased mutational burden, such as breast, prostate, cervical, neuroendocrine cancers, and sarcoma. This massive dataset further allowed the authors to chart the functional impact of DNA polymerases *Polε* and *Polδ1* mutations at fine resolution. It also enabled the application of a personalized genomic approach for identifying patients who may require family screening for inherited DNA repair syndromes, as well as expanding the use of immunotherapy, thought to be particularly effective in cancers with an increased mutational burden.

Notably, the little negative selection shown by the dN/dS analysis may suggest that mutations leading to strongly immunogenic neoantigens are a rare occurrence in the natural evolution of cancer (outside the context of immunotherapeutic interventions). In contrast, the dN/dS lens highlights *B2M* and *HLA* genes, crucial for effective adaptive immunity, as targets of positive selection in stomach, lung, colorectal, and head-and-neck cancers. We speculate that these cancers evolve to disrupt anti-tumor immunity, as they may be otherwise particularly immunogenic due to oncogenic virus integration or due to a high mutation burden that leads to the emergence of effective neoantigens. Collectively, these analyses provide rare and intriguing insight into the activity of immune surveillance in the early stages of clonal transformation.

A potential future development of these studies would be to explicitly account for the clonal complexity within tumors (Landau et al., 2013; McGranahan et al., 2016). The current dN/dS analysis treats the cancer genome as a single lineage, compared to the parental germline lineage of the patient. This framework lumps together the entire evolutionary history of the cancer into a single process, whereas many somatic mutations precede the malignant transformation. It may be worthwhile considering that the selection forces shaping the cancer genome prior to and during the malignant transformation may differ from the ones operating in a clonally diverse growing malignant population. For example, frequent clonal convergence, leading to independent acquisition of similar driver events within a single cancer, may modify the positive selection estimates. An analysis accounting for intratumoral clonal diversity will likely also add further nuance to the estimates of the number of driver mutations per cancer, as the current estimates may reflect drivers in independent subclones.

Moreover, recent evidence suggests that immune surveillance also acts differentially on clones, leading to purifying subclonal selection (Zhang et al., 2017).

Another epoch of cancer evolution worthy of further exploration involves our understanding of how therapeutic intervention shapes the cancer genome. Effective anti-neoplastic therapies apply strong negative and positive selection pressures on the growing tumor, which change depending on the therapeutic class. While Martincorena et al. studied primarily untreated tumors in the TCGA collection, Campbell et al. showed that in many cancers, prior alkylating agent therapy led to a dramatic increase in mutation burden, which may potentiate immunotherapeutic approaches.

Collectively, Martincorena et al. and Campbell et al. herald a new phase of maturity for cancer genomics. The search for cancer genes, at least in the coding genome, is coming to a close. In its stead, vast datasets now allow us to define the fundamental evolutionary principles that drive cancer and break open new fields of investigation that will enhance not only our understanding of tumor biology but also of how cancer genomics can be personalized for therapeutic impact.

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Tuning Biased GPCR Signaling for Physiological Gain

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https://doi.org/10.1016/j.cell.2017.10.046

Effective and safe doses of opiate painkillers, like morphine, can be limited by respiratory depression. Schmid et al. (2017) now present a quantitative method to design ligands and correlate GPCR signaling bias to the dose separation between therapeutic and adverse effects in animals.

Overdose deaths from prescription opioids have quadrupled since the late 90's (National Academies of Sciences, Engineering, and Medicine, 2017). Furthermore, those dependent on opioids, including chronic pain patients, are at higher risk for overdose due to slower development of tolerance to respiratory depression compared to analgesia (Boyer, 2012). Therefore, it is important to increase the therapeutic window of opiates by creating drugs that maintain the ability to relieve pain at doses that do not impact respiration. Opiates target μ-opioid receptors (MOR) in the brain stem and spinal cord to cause analgesia. Brain stem γ-opioid activation also results in respiratory depression, which can lead to death. As a canonical Gi-coupled G-protein-coupled Receptor (GPCR), MOR signals primarily through two downstream cascades: those initiated by G proteins and those by arrestin scaffolding. Previous studies have suggested that "biased" molecules that preferentially engage and stabilize MOR conformations selective for specific pathways downstream are potentially less likely to have adverse side