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SHORT REPORT

The (not so) immortal strand hypothesis

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Abstract

Background: Non-random segregation of DNA strands during stem cell replication has been proposed as a mechanism to minimize accumulated genetic errors in stem cells of rapidly dividing tissues. According to this hypothesis, an "immortal" DNA strand is passed to the stem cell daughter and not the more differentiated cell, keeping the stem cell lineage replication error-free. After it was introduced, experimental evidence both in favor and against the hypothesis has been presented.
Principal findings: Using a novel methodology that utilizes cancer sequencing data we are able to estimate the rate of accumulation of mutations in healthy stem cells of the colon, blood and head and neck tissues. We find that in these tissues mutations in stem cells accumulate at rates strikingly similar to those expected without the protection from the immortal strand mechanism.
Significance: Utilizing an approach that is fundamentally different from previous efforts to confirm or refute the immortal strand hypothesis, we provide evidence against non-random segregation of DNA during stem cell replication. Our results strongly suggest that parental DNA is passed randomly to stem cell daughters and provides new insight into the mechanism of DNA replication in stem cells.

Introduction

Before a cell divides, its DNA content is replicated and then segregated during mitosis. Whether the DNA strands are passed randomly to the two daughter cells or according to some other mechanism is however not clear.

The immortal DNA strand hypothesis has been proposed (Cairns, 1975) as a mechanism used by stem cells in order to minimize the accumulation of mutations in their genomes. This hypothesis proposes that stem cells divide predominantly asymmetrically, producing one stem cell and one differentiated cell, and that at each division, a template set of DNA strands (known as the "parental" or "immortal strands") is transferred to the daughter stem cell and not to the non-stem cell daughter. The fundamental idea behind this hypothesis is that, by retaining the immortal strands within the stem cell progeny, the accumulation of random DNA replication errors in the stem cell compartment would

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be greatly reduced, therefore decreasing the risk of genetic disorders such as cancer. Indeed, errors in DNA duplication would be passed on to non-stem, more differentiated, and shorter-lived daughter cells.

The earliest experimental evidence for the non-random segregation of stem cell DNA strands in mammalian tissues comes from studies by Potten et al. of tongue epithelia and intestinal crypts (Potten et al., 1978). Although some further experimental work appears to support this hypothesis (Potten et al., 2002; Smith, 2005; Karpowicz et al., 2005; Shinin et al., 2006; Conboy et al., 2007; Armakolas and Klar, 2007), whether such a mechanism is operational in adult stem cells in vivo or not is still controversial (Haber, 2006), with reports arguing against the existence of immortal strands in intestinal crypts (Steinhauser et al., 2012) and hematopoietic stem cells (Kiel et al., 2007). Most of the studies arguing for or against the immortal strand relied on labeling early stem cells and testing whether segregation of label followed a random or non-random pattern. Here we use a different approach that utilizes genetic sequencing data and provide new evidence against the immortal strand hypothesis, by showing that stem cells in hematopoietic, colorectal and head and neck epithelial tissues contain as many somatic mutations as would be expected if no protection mechanism were in place.

Results

Tomasetti et al. (2013). recently provided a new methodology for estimating the number of mutations that accumulate in healthy self-renewing tissues. Their results were obtained via the analysis of The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) whole-exome sequencing data on somatic mutations from patients with a given tumor type. Tomasetti et al. found statistically significant strong positive correlations between age and number of somatic mutations in tumors of several self-renewing tissues, in patients with matched tumor stages (Fig. 1A). For example, the data show that colorectal cancers from 85-year-old patients have, on average, 47 mutations more than colorectal cancers at the same stage from 45-year-old patients. Since the cancer stage is the same, those extra 47 mutations are, on average, not due to the cancer phase, but to the normal accumulation of somatic mutations occurring in the healthy tissue during the extra time the older patients had before tumorigenesis started (Fig. 1B). This allows the estimation of the rate at which mutations accumulate in healthy cells, prior to the first driver mutation hit. This method represents a unique way of indirect single cell sequencing, since all tumor cells carry the changes present in their last healthy ancestor in addition to changes accumulated during tumor evolution (Fig. 1B). Importantly, their results yielded estimates for the rate of accumulation of somatic mutations in healthy tissues that are remarkably in line with estimates obtained via completely different methodologies (Tomasetti et al., 2013).

The first initiating event that starts the process of tumorigenesis must originate in a healthy cell. It is possible that this healthy cell may not be a stem cell (O'Brien et al., 2007). However, given any cell of a healthy self-renewing tissue, the number of divisions that separates this cell from its mother stem cell is not very large. And even a hundred divisions will not create any relevant difference between the mutational load of the stem cell mother and its healthy differentiated daughter cell. In fact, normal somatic mutation rates are of the order of 10^{-10} per base per cell division (Tomasetti et al., 2013), and therefore 100 divisions will not cause, on average, even one extra point mutation in the exome of the differentiated cell. Thus, we can safely assume that the mutational load found in healthy cells is an accurate estimate for the mutational load of their mother stem cells.

We are then able to provide estimates for the number of somatic mutations accumulating in healthy stem cells of self-renewing tissues as a function of time. In Fig. 2A, we show the average number of somatic mutations accumulating with age in stem cells of the colonic crypt (red line), plus or minus 2 standard errors (shaded red area), as it was estimated from TCGA sequencing data in Tomasetti et al. (2013). We next compare these estimates with the number

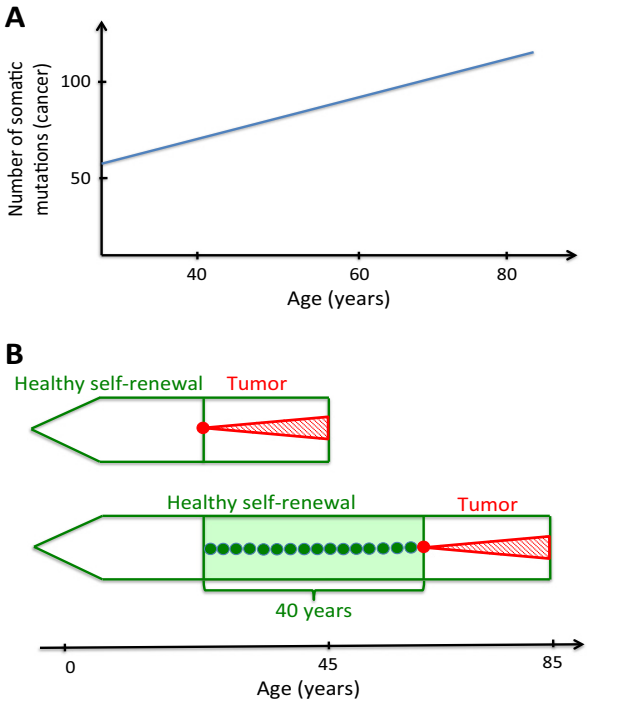

Figure 1 Number of mutations in a patient's tumor is a function of age. A) Tomasetti et al. (2013) found that the number of mutations in cancer tissues correlates significantly with the age of the patient in chronic lymphocytic leukemia, uterine corpus endometrioid carcinoma and colorectal cancer, independent of the cancer stage. For example, colorectal cancers in 85-year olds harbor on average 121 mutations, in contrast to 74 mutations in colorectal cancers of 45-year old patients. B) Difference in the numbers of somatic mutations detected in the cancers of patients of different ages can be explained by the difference in the length of the self-renewal phase in the lineage that lead to cancer. Thus the average difference in the numbers of mutations in colorectal cancer patients aged 85 and 45 provides a good estimate for the number of somatic mutations that accumulated during 40 years of healthy self-renewal. This allows us to infer the normal rate at which mutations accumulate in healthy stem cells.