



Cancer-like mutations in non-cancer tissue: towards a better understanding of multistep carcinogenesis

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Using Duplex Sequencing, the most accurate sequencing technology currently available, we recently reported the novel finding that mutations in TP53, the most commonly mutated gene in cancer, are present at a very low frequency in non-cancerous tissues of women with and without ovarian cancer (1). These mutations were mostly non-synonymous, clustered in common TP53 cancer-associated hotspot codons and frequently led to an inactive protein. Thus, the mutations were not simply random, but appeared to have arisen through positive selection, akin to that occurring in tumors, yet in normal tissue. In most patients, multiple “biological background” mutations were simultaneously present, and mutation abundance increased with patient age. Taken together, our observations support the striking conclusion that clonal evolutionary processes typically thought of as operative only in neoplasia are, in fact, a ubiquitous part of normal aging.

This finding has profound implications for efforts to develop early cancer detection tests based on deep sequencing of cancer genes in liquid biopsies. An excellent recent Editorial by Drs. Oien and Chien based on our article discusses this challenge in the context of ovarian cancer screening (2). An eloquent Commentary by Dr. Sicotte discusses the greater accuracy of Duplex Sequencing compared to other error-correction technologies, and the broader implications that improved sensitivity may have on cancer specificity (3). In both of these valuable discussions of our work, we especially appreciate the insightful remarks about the critical need to better understand the biology of background mutations in order to distinguish them

from those which are cancer-derived. Until recently, our collective knowledge of how somatic mutations accumulate in normal tissue was limited. However, this is changing quickly with the advent of more powerful sequencing technologies. We take this opportunity to highlight some of the recent landmark studies, which are likely to be just the tip of an emerging iceberg.

The rate of somatic mutation in normal tissue has been much debated, but it is clear that mutations that do arise are typically confined to a relatively small number of cells, in contrast to the large clonal expansions in tumors. This significantly hindered their detection until the advent of next-generation sequencing (NGS), which can resolve smaller subclones than Sanger methods. Three large population studies using NGS were published in 2014 reporting the presence of clones carrying leukemia-associated mutations in the blood of ~10% of healthy individuals older than 70. A follow-up study this year using an error-correction approach that enabled the detection of rarer mutations found these same types of clones in almost all (95%) 50–60 years old (4). Another study using an NGS-based analysis of somatic mutations in microdissected pieces of sunlight exposed skin revealed positively selected mutations in as many as a third of normal skin cells (5). Very recently, a low-coverage genome-wide application of Duplex Sequencing revealed age- and tissue- dependent accumulation of mutations in normal tissue, which resembled the mutational pattern seen in cancers of the same organ. Mutation burden increased with carcinogen exposure and inherited DNA repair deficiency (6). Very

similar results were obtained in a previous study that analyzed TCGA paired benign tissue and blood (7).

A detailed analysis of clonal mutations present in tumors has also provided recent new insights into mutational events that occur in normal cells prior to their transformation into cancer. Because cancers are clonally derived, clonal mutations in tumors encompass both the normal age-associated mutations present in the original cell and those which accumulated during cancer progression. In most cancers, the burden of clonal mutations is associated with the age of the individual at diagnosis. This has been used to estimate that about half of cancer mutations originate prior to tumor initiation (8). Analysis of TCGA data revealed that age-related mutations accumulate at different rates in different tissues and can be classified into two distinct signatures (9,10). Little is known about the biological processes that underlie these signatures, other than one appears related to deamination 5-methylcytosine at CpG nucleotides and is associated with rate of cell division.

Although there are still many gaps in knowledge, the emerging picture from these studies and our own is that low frequency somatic mutations are widespread in normal tissue, accumulate with age, and fuel clonal expansions under the influence of positive selection. This process contributes to an ever-increasing pool of clones within otherwise normal tissue, among which malignant transformation can take place. Even though clones carry mutations in cancer-associated genes, they rarely progress to malignancy, presumably because of the action of tumor suppression mechanisms.

This model of carcinogenesis has major implications for early cancer detection using liquid biopsies. Extremely accurate sequencing technologies deliver increased sensitivity, but deeper sequencing is bound to reveal cancer-like, biological background mutations, which seriously compromise specificity. Whether the tumor-specific mutation can be detected above the biological background for a given individual depends on multiple factors. Collectively, these recent studies show that the mutational background differs by age, tissue type, environmental exposures, and inherited genetic makeup (1,4,6,7). Thus, these factors need to be taken into account in experimental designs, which will require cautious application of sequencing technologies as well as meticulous comparison with appropriate cancer-free control groups. In situations in which the biological background is problematic, a variety of strategies could be developed to enhance disease specificity. These include testing for combinations of

mutations, especially those that may be more specific of malignant transformation; use of quantitative thresholds; and interrogation of longitudinal biopsies.

Beyond early cancer detection, assessment of mutational burden could be informative to assess cancer risk. Those with an increased number of expanded clones might be at a higher risk of cancer progression, or perhaps even shorter longevity. Underlying these translational possibilities is a more fundamental biological question: how does a cancer clone escape the mechanisms that keep innumerable other expanded clones from malignant progression? The answer is likely to be complex, multifaceted, and tissue specific. The recognition that these age-associated, cancer-like mutant clones exist and evolve over a lifetime is the first step on the long, and undoubtedly fascinating, path ahead.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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