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Letter to the Editor

Reply: Is there any genetic instability in human cancer?

This letter is in response to the recent communication of Darryl K. Shibata and Michael R. Lieber arguing against the concept of a mutator phenotype in human cancer [1]. The question is whether an increased mutation rate is required to account for the large numbers of mutations observed in cancer (i.e. a mutator phenotype) or whether normal mutation rates are sufficient [2]. They base their argument on the recent report of 33,000 genetic changes per genome between a tumor cell line and a paired nonneoplastic cell line [3]. They calculate that a tumor cell could divide once every day and, assuming a normal mutation rate of 10^{-9}, an individual might accumulate "60,000 mutations per haploid genome by middle age." Calculations of this nature are highly speculative, in that estimates of all the relevant parameters can vary over several orders of magnitude.

Experimental estimates of the number of cell divisions in cancers have been understandably difficult to ascertain. Cell lines are the products of selection, and their division and mutation rates may not correlate with those exhibited by normal and malignant cells. Vogelstein and colleagues have proposed >5000 generations as an estimate of the number of sequential cell divisions that might occur prior to the last clonal expansion in colon cancers [4,5] and Shibata and Lieber now suggest 20,000. While rapid sequential divisions and extensive tumor cell death are easy to visualize for tumors that line a body cavity such as the colon, it is much more difficult to visualize or substantiate in slow growing solid tumors encapsulated by normal tissue. Estimates of the number of cell divisions per human lifetime range as low as 150–200 cell generations per lifetime for non-stem cell compartments [6–8], compared to the 20,000 that are postulated by Shibata and Lieber.

More importantly, we are now in possession of solid estimates of mutation frequencies in normal human cells and in several solid cancers [9]. Roach et al. [10] sequenced the whole genomes of a family of four, consisting of two siblings and their parents. They found that only 70 mutations were identified between generations. They directly estimated a human intergeneration mutation rate of 1.1×10^{-8} per nucleotide position per haploid genome. (Based on 100 cell divisions, the mutation rate per cell generation would be 1×10^{-10} per nucleotide position compared to the 10^{-9} considered by Shibata and Lieber; with a larger number of cell divisions it would be even lower. Estimates of mutation rates in cultured cells range as low as 10^{-11} [11].) In contrast, genome-wide sequencing of breast, melanoma and lung cancer genomes each identified >20,000 clonal mutations per cancer genome [3,12,13]. A mutation load of >20,000 would therefore require an amount of proliferation equivalent to that of almost 300 human generations!

We agree with the statement of Shibata and Lieber that cancers arising within a human lifetime "take the fastest pathways", since all possible mechanisms are likely in competition. If "the mutations that cause cancer are likely due to a few unlucky critical hits," as they assert, what is the most efficient and fastest way to obtain these critical hits? Extensive quantitative modelling has shown the most efficient trajectory for a tumor is to sustain an early mutator mutation, and that this is true irrespective of other complications such as lineage selection and expansion, and for nearly all reasonable parameter values [14–16]. These critical mutations represent a subset of the clonal sequence changes. Because these cells preferentially have an increased overall mutation rate, additional random mutations also occur as a consequence. Even when these random mutations are not drivers of carcinogenesis, they may contribute to plasticity in response to therapy.

The defining feature of the cancer genome is the extensive genomic heterogeneity generated during tumorigenesis, both within individual tumors and between different tumors of the same type. Shibata and Lieber rightly point out that the mutational heterogeneity between tumors complicates the usefulness of sequencing large numbers of cancer genomes. Furthermore, we argue that substantial mutational diversity also exists within individual tumors. Attempts to characterize the cancer genome through large-scale sequencing studies continue to ignore that it is the generation of mutational diversity itself (i.e. the expression of a mutator phenotype), combined with multiple rounds of selection, that generate the phenotypic plasticity required for tumorigenesis [17,18]. To use DNA sequencing for selecting individual cancer therapies (personalized medicine) or for predicting the likelihood of emergence of resistance, it may ultimately be necessary to quantify the heterogeneity of tumors by sequencing DNA from single cells or molecules.

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Edward J. Fox [a]
Robert A. Beckman [b]
Lawrence A. Loeb [a,*]
[a] *Department of Pathology, University of Washington, K-072 HSB, Seattle, WA 98195-7705, United States*
[b] *Department of Oncology Clinical Research, Daiichi Sankyo Pharmaceutical Development, 399 Thornall Street, Edison, NJ 08837, United States*

* Corresponding author. Tel.: +1 206 543 6015; fax: +1 206 543 3967.
E-mail address: laloeb@u.washington.edu (L.A. Loeb)

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