Is the evolution in tumors Darwinian or non-Darwinian?

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Although Darwinian selection driving the genetic diversity within tumors has been widely assumed by the field of cancer biology, recent studies that employ the rigorous tests of population genetics have increasingly suggested otherwise. In these new studies [1–5], the non-Darwinian, or neutral, model of evolution can adequately explain the documented diversities in tumors without invoking natural selection. Therefore, the half-century-old debate of selectionism vs. neutralism [6–8] on the role of natural selection in populations is being replayed at the cellular level. The debate will have clinical relevance because the level of genetic diversity in tumors depends strongly on whether and how natural selection operates [1–5]. This diversity may in turn influence tumors’ capacity to respond to medical interventions. It should be noted that, although selection may well be the main force driving cancer formation, the diversity within tumor is not necessarily selectively driven. An analogy is that the diversity within human populations may be largely neutral while the divergence between human and chimpanzee may have been extensively selected.

The two studies that are most explicit about non-Darwinian evolution in tumors take very different approaches [2,4]. Ling et al.’s approach to testing Darwinian evolution is to obtain a large number of samples from a single tumor (n = 286 in a hepatocellular carcinoma [HCC] tumor), which are used to infer the operation of natural selection in the entire cell population. Its power to reject neutrality at the whole-tumor level is demonstrated by a hypothetical example where only one single polymorphic mutation is driven by selection (to a frequency of 90%). With the power, they concluded that the HCC tumor follows the null model of neutral evolution. It is interesting to note that, in several other HCC cases with less dense sampling, Tao et al. (2015) [5] failed to reject neutrality even though it has the power to reject the null model in inter-tumor comparisons. In short, the statistical power (1 – β where β is type II error, or the probability of incorrectly accepting the null model) to reject neutrality is crucial in intra-tumor studies.

In a recent study, Williams et al. used one sample each from 904 tumors to test each sample individually for neutrality. They asked whether the polymorphism within each sample follows the predicted pattern of neutral evolution and concluded that at least one-third of the samples do. These two studies [2,4] examined two different stages of a single process—the low- vs. high-frequency stage. Williams et al.’s study tends to be local, pertaining to small aggregates of cells, whereas Ling et al.’s approach is global, pertaining to the evolution of the entire tumor. Although the two stages are parts of the same continuous process, they provide very different glimpses. An analogy is local human populations where one often finds disease-causing mutations that are extremely rare in global populations [9]. The opposite is also true—advantageous mutations may be highly polymorphic globally but are monomorphic at the local level (where such mutations are either nearly 100% or 0%) [9].

In analysing the large number of local samples of The Cancer Genome Atlas (TCGA) project, the issue would be the power of local sampling in detecting selection. As stated above, when a good mutation rises to 90% in the global population, it should be fixed in most local samples and would be excluded from the analysis. On the contrary, mutations polymorphic in a local sample may be present in only a fraction of cells in the entire population as cells within a solid tumor do not appear to move freely [2,4]. For example, a mutation that accounts for 10% of a local sample, which is itself 1% of the tumor mass, might be in the frequency range of 0.1–1% of the population. We now ask whether selection can be detected in the low-frequency range by using one local sample only.

We first consider cell populations that are expanding, as such expansion can substantially complicate the detection of selection. Depending on the growth parameters, the frequency spectrum of mutations may vary greatly even without selection. With selection, the frequency spectrum could then resemble a neutral spectrum under the same or a different growth mode, be it exponential, quadratic, indolent or growth interspersed with indolence. In Ling et al.’s [2], four different models of neutral tumor growth were considered and each made a different prediction. Since Williams et al. used an exponential model, we simulated the frequency spectra by the same branching process to illustrate the
Figure 1. The frequency spectrum of mutation under different strengths of selection, displayed as increasingly darkened red color. The $X$-axis is the frequency of mutation in the entire cell population and the $Y$-axis is the (relative) number of mutations with frequency $x$. Both axes may be in the log scale to display the key portions of the graph. (a) Mutation spectra in growing populations simulated by the branching process. In every time interval, each wild-type cell produces $n$ progeny, which follows a Poisson distribution with the mean of $\lambda_w$ ($w$ for wild-type). Similarly, the mutant progeny mean is $\lambda_m$. When either $\lambda$ is greater than 1, the population grows. We note that the growth rate of the neutral case (the blue line; $\lambda_w = \lambda_m = 1.01$) is between the two populations under selection (the red lines; $\lambda_m = \lambda_w + 0.01$ in both cases), suggesting the lack of power to reject neutrality in non-stationary populations. (b) Mutation spectra in stationary populations according to Eq. (1). The maximum on the $Y$-axis is scaled to 1 and the selective intensity ($2Nes$) ranges from –1 to 100 and beyond as indicated. The curves with different $Nes$ values begin to diverge only when $x > 0.1$. This is true even when $Nes$ is implausibly high. In the inset, the $X$-axis is given in the log scale to highlight the low-frequency portion of the spectra. The range of $x = (0.01 – 0.1)$ marked by an oval may correspond to the population frequencies of locally identified mutations. The overlapping curves of different colors suggest the lack of power in discriminating frequency spectra under different selection intensities.

The challenge in dealing with populations of non-constant sizes. Fig. 1a shows two predictions with the same selection intensity but different growth rates (red lines). In between them is the neutral spectrum when the population is growing at the intermediate rate (blue line). Since growth rate could influence the observations as much as selection could, it would be virtually impossible to distinguish between selection and neutrality when the growth parameters are not known. This is especially true when one cannot inspect high-frequency mutations, which are usually fixed in local samples.

Given the difficulty, the condition for rejecting neutrality in favor of selection is when the growth parameters are known; hence, zero (or near zero) growth is the best condition for such a test. We now ask whether, and when, local samples could offer the power to reject neutrality under the most favorable condition. With constant population size, the frequency spectrum of polymorphic mutations driven by selection is given by

$$
\Phi(x) = \frac{\theta (1 - e^{-\sigma(x-1)})}{(1 - e^{-\sigma})x(1 - x)}.
$$

Eq. (1) is the density function of mutations [10] at the frequency $x$ with $\theta = 2N_e\mu$ ($N_e$ being the effective population size and $\mu$ being the mutation rate) and $\sigma = 2Nes$ ($s$ being the selective coefficient). Fig. 1b presents the frequency spectra by Eq. (1) under different selection intensities. These trajectories express relative mutation numbers at different frequencies and are independent of $\theta$. It is clear that the relative number of mutations with a frequency below 10% in the entire tumor would be nearly unaffected by selection. The inset of Fig. 1b provides a close-up view of this property. In comparison, the high-frequency portion of the spectrum does provide some resolution of differences between selection and neutrality.

It seems clear that, regardless of how fast tumors grow, there is no substitute for dense sampling when attempting to address the issue of selection. The field of molecular evolution in the last half-century is instructive[6–8] about the challenges of settling the neutralism–selectionism debate. The answer is crucial to the long-term evolution as well as the short-term polymorphism in populations. Recent studies[1–5] clearly show the need to introduce population genetic analyses and to increase the breadth of sampling in studying tumor evolution. These studies prudently suggest the null neutral model as the appropriate explanation for the observed intra-tumor diversities. Selection, on the other hand, is a postulate that will need rigorous proof. These publications also gave several reasons for the possible low efficacy of selection within tumors.
Evolution, unlike development, does not follow a pre-determined pattern. Hence, each case of cancer will require the detailed treatments applied to the studies of one single species. Broad surveys can then build on many well-sampled case-by-case analyses.

While Williams et al.’s approach [4] may not be able to distinguish between neutral and selection-driven tumor growth, their analysis has been very informative about the mode of tumor growth. They found that two-thirds of cases are incompatible with the neutral exponential growth model. These cases suggest that, even at the local level far below the ‘carrying capacity’, the growth dynamics often deviates from the exponential mode that is commonly assumed. Finally, studies of tumor evolution have generally followed models developed for natural populations. A recent study [11] has proposed a more general approach to random genetic drift than the conventional Wright-Fisher model of spatial panmixia [6,7,10]. This ‘individual output’ model may be more suitable for modeling tumor evolution than practiced so far.

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On 27 April 2017, NG offered acceptance conditional on the removal of the mathematical modeling section, which makes assumptions about cell population size, N. On 2 May 2017, we replied that ‘we demonstrate the lack of power [in Williams et al.’s model] when the power is the highest possible, i.e., when N is constant. If the data used could not reject neutrality when all variables are most conducive for rejection, then the test has no power under all circumstances.’ On 4 May 2017, NG replied that keeping the mathematical section would require two more months of further reviews. On 12 May 2017, this critique was withdrawn from NG. We welcome continual exchanges on a forum independent of the journal that published the original article.

We fully agree with Williams et al.’s conclusion that the neutral model should be considered the null hypothesis in the study of tumor evolution. These exchanges have hence helped to strengthen this position. We only wish to clarify the procedure of hypothesis testing. An overly simple model, such as Eq. (1) of our analysis, is useful as a gauge of the power of statistical testing because simple null models should be relatively easy to reject. For that reason, a test that fails to reject the simple model, when the model is wrong, may not have adequate statistical power. Eq. (1) was employed because it is the simplest stochastic model with selection.

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Molecular Biology & Genetics

Reply: Is the evolution of tumors Darwinian or non-Darwinian?

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Wu and colleagues’ letter discusses the limitations of the use of a single sample per tumor to investigate neutral evolution in human cancers. Neutral tumor evolution describes the situation in which there is no differential clonal selection amongst the population of cells within a cancer: all mutations that accrue during growth are passengers and all drivers were already present in the most recent common ancestor of the population.