LETTER

Constrained positive selection on cancer mutations in normal skin

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Despite years of extensive cancer genome sequencing, very little is known about the extent of somatic mutation and selection in normal tissues. To address this, we recently performed a sequencing study of 234 small biopsies of normal skin from four middle-aged healthy individuals (1). This study revealed that cells from sun-exposed skin carry many thousand somatic point mutations, a similar number to many cancers. Furthermore, we showed that mutations in several cancer genes are under strong positive selection in the skin, as demonstrated by rates of nonsynonymous mutations (dN) being many times higher than expected from synonymous mutations (dN/dS>>1, often >10).

In a very interesting paper in PNAS (2), Simons reanalyzes our data on clone sizes and shows that the size distribution of mutant clones is largely consistent with clones growing by neutral drift. His analysis suggests that most mutations are neutral, with only a small minority of clones being larger than expected by neutral drift. This finding is also consistent with our own original observations that clones carrying driver mutations do not appear to be much larger than those without them (1). Simons notes that this is paradoxical (2), with genetic analyses demonstrating strong positive selection but clone sizes supporting neutral clonal growth.

We would like to clarify that both observations are not incompatible and are indeed complementary. It is important to note that our sequencing assay was only sensitive to clones larger than several hundred cells. The analysis of clone sizes by Simons (2) is thus only informative about selection acting on clones large enough to be detectable. In contrast, the analysis of selection using the rates of nonsynonymous vs. synonymous mutations (dN/dS), from all detected mutations, is only informative about selection acting when clones are smaller than our detection limit. The large excess of nonsynonymous mutations that we reported in several genes (including *NOTCH1–3*, *FAT1*, and *TP53*) reveals that these cancer-driver mutations largely increase the probability of mutant clones reaching detectable sizes. In contrast, the sizes of observable clones suggest that the clonal advantage conferred by these mutations does not seem to continue as clones grow larger. Taking these data together, as we explained (1), both observations suggest that positive selection on driver mutations is strong only during the initial expansion of mutant clones.

Importantly, this finding is also consistent with observations of p53-mutant clones detected by immunostaining in human skin and Notch-mutant clones in mouse esophageal epithelium (3–5). In both examples, initial exponential growth is followed by reversion to neutral drift, a change accompanied by crowding of proliferative mutant cells. Such clonal "imprisonment" may arise from physical constraints limiting the expansion of clones or density-dependent induction of differentiation (6–8). Whatever the mechanism, constraining the rapid growth of clones carrying cancer-driver mutations must be critical in any tissue to limit the incidence of cancer. This is patent in normal sun-exposed skin: even though selection leads to relatively small (typically submillimetric) clones, this is sufficient to greatly increase the frequency of driver mutations in normal skin, with a quarter of all cells carrying cancer-driver mutations (1).

1 Martincorena I, et al. (2015) Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348(6237):880–886.

2 Simons BD (2016) Deep sequencing as a probe of normal stem cell fate and preneoplasia in human epidermis. *Proc Natl Acad Sci USA* 113(1):128–133.

3 Zhang W, Remenyik E, Zelterman D, Brash DE, Wikonkal NM (2001) Escaping the stem cell compartment: Sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci USA* 98(24):13948–13953.

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4 Alcolea MP, et al. (2014) Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change. *Nat Cell Biol* 16(6): 615–622.
5 Klein AM, Brash DE, Jones PH, Simons BD (2010) Stochastic fate of p53-mutant epidermal progenitor cells is tilted toward proliferation by UV B during preneoplasia. *Proc Natl Acad Sci USA* 107(1):270–275.
6 Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci USA* 102(9):3318–3323.
7 Eisenhoffer GT, et al. (2012) Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature* 484(7395):546–549.
8 Watt FM, Jordan PW, O'Neill CH (1988) Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc Natl Acad Sci USA* 85(15):5576–5580.