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Buckle up! Transposon mutagenesis can differentiate melanoma drivers from their many passengers

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BRAF^{V600E} mutations are found in ~50% of cutaneous malignant melanomas and drive cancer progression through constitutive activation of the RAS–RAF–MAPK pathway. The discovery of BRAF^{V600E} over a decade ago revolutionized melanoma research; for the first time, the field had a therapeutic target for small-molecule intervention. The majority of patients treated with the BRAF^{V600E} inhibitor vemurafenib demonstrate remarkable and almost complete remission; however, this initial response is nearly always followed by a fatal relapse. Although oncogenic BRAF is a driver of melanoma, alone it is insufficient for tumorigenesis and leads instead to senescence-like Growth arrest. Nevi harboring BRAF^{V600E} progress to malignancy only after accumulating additional mutations, which cooperate with oncogenic BRAF to promote melanomagenesis. Known BRAF^{V600E} cooperating mutations include those in the tumor suppressor genes (TSGs) INK4A, ARF, PTEN, and TP53; however, detailed genomic analyses revealed extensive tumor heterogeneity, suggesting that many more cooperators exist.

Unfortunately, identification of these cooperators is far from trivial. Human melanocytes, which constitute the melanoma cell-of-origin, function to protect the body from DNA-damaging UV irradiation and can withstand high levels of genotoxic stress. It is therefore unsurprising that melanoma has the greatest mutational load of any cancer (Hodis et al., 2012). This high frequency of genomic alterations confounds the discernment of events which confer selective advantage to cancer progression, termed the drivers, from background passenger mutations, which confer no apparent advantage. Mouse models of human cancer can help distinguish passenger mutations from driver mutations, and complement human tumor sequencing experiments. The identification of homologous mutations in both mouse and human cancers strongly supports the contention that such mutations have occurred in drivers that convey a selective advantage to the cancer cell. However, due to a well-populated mutational landscape, melanoma models that employ chemical- or UV-induced mutagenesis require sequencing of many samples to identify significant drivers. Furthermore, other models that employ genetic engineering of multiple known driver alleles produce excessively fast-developing melanomas that fail to recapitulate the genomic diversity characteristic of human melanoma progression, which occurs over decades.

In this year's March issue of *Nature Genetics*, Mann et al. used a transposon-mediated mutagenesis approach to introduce a high-copy number of traceable, random mutational events, thereby eliminating the need for deep sequencing. Developed in 2005 for germline mutagenesis (Keng et al., 2005) and then successfully employed in cancer studies (Dupuy et al., 2005; Moriarity and Largaespada, 2015), *Sleeping Beauty* (SB) mutagenesis relies on the

random genomic integration of the SB transposon in the presence of transposase. The SB construct can either disrupt or activate genes into which it is incorporated; bidirectional polyadenylation sequences induce sequence disruption while a unidirectional enhancer and promoter induce sequence up-regulation, allowing for the identification of both putative TSGs and oncogenes (Moriarity and Largaespada, 2015). This system allows for rapid and inexpensive mapping of insertions and, through directionality of the transposon, can predict whether insertions occur within TSGs or oncogenes. The authors employed whole-exome sequencing to confirm that SB was the main cause of mutation in their model and also successfully demonstrated that the mutational load in their system is relatively low when compared to UV mutagenesis, thus reducing the sample sizes necessary to differentiate driver from passenger.

To specifically identify BRAF^{V600E} cooperators, Mann et al. expressed both inducible BRAF^{V600E} and transposase in the mouse melanocyte lineage. In this way, they were able to restrict SB mutagenesis to melanocytes expressing BRAF^{V600E}, thereby allowing detection of additional genetic events that co-drive tumor progression. In total, Mann et al. sequenced 77 melanomas and identified 1,232 common transposon insertion sites (CIS) (Moriarity and Largaespada, 2015). Of these, more than 95% were predicted to be TSGs, which are notoriously difficult to target by therapeutic intervention and usually require modulation of downstream/parallel effectors in TSG signaling networks. As such, for clinical translation it is paramount to understand the functional networks of the TSGs identified. Using informatics resources, the authors were able to demonstrate statistically enriched functional connectivity between CIS genes within all tumors, including known melanoma networks such as PKA, Wnt/ β -catenin, and MAPK signaling cascades. Using cross-species analysis, 46 genes were significantly associated with patient survival, and 12 of these were highly connected in functional networks, which the authors postulated would make them promising candidates for future molecular biology analyses. While Mann et al. successfully identified common networks within all tumors, the authors did not address how these networks might interact with one another to aid progression within a given melanoma, a critical issue for targeted therapy design. Future studies would benefit from network analysis on a tumor-by-tumor basis to address which combinations of signaling perturbations promote melanoma progression.

A major limitation of whole tumor sequencing approaches is the inability to distinguish early from late mutational events. Early events are likely to be crucial and are presumably the most effective targets for therapeutic intervention. In addition, the only current criterion for monitoring nevus-to-melanoma progression in patients is crude observation of the physical appearance and size of nevi. Early genetic markers would allow more robust prevention and screening. By mapping CIS and further selecting the most prevalent CIS based on the number of sequencing reads, representing clonal selection of CIS, Mann et al. were able to predict 21 melanoma early progression genes. Reassuringly, of these two were known melanoma progression genes and three were known cancer progression genes. To further validate this gene cohort, they showed that shRNA knockdown of the highest ranked novel CIS gene, Cep350, promotes tumor progression in xenograft models. Mann et al. successfully filtered their gene set for the most abundant CIS to identify putative early melanoma progression genes; future analysis can now address how perturbing any of these

gene pathways could contribute to progression of BRAF^{V600E}-driven melanoma, a key question that needs to be answered for translation to the clinic.

This study displayed a strong bias toward discovery of putative TSGs, most of which were predicted to be haploinsufficient. Such mutations have been challenging to identify in human sequencing studies due to the high mutational load of melanoma (Hodis et al., 2012), suggesting that high-copy SB mutagenesis could be an effective method to screen for haploinsufficient TSGs. By employing a strong oncogenic driver such as BRAF^{V600E}, the authors may have biased the study toward the discovery of TSGs; combining the SB mutagenesis system with other drivers could broaden the scope of identifiable cooperating mutations. However, previously Ni et al. (2013) used low-copy number transposon mutagenesis (the piggyBac approach) in BRAF^{V600E}-transformed melanocytes to successfully identify 25 putative proto-oncogenes (66% of all genes identified). Ni et al. (2013) postulated that the use of a low-copy number approach circumvented the need to select candidate genes by mutational frequency, which could bias a study toward non-specific non-sense/ frame-shift mutations, and subsequently TSG candidate gene discovery. Thus, transposon copy number could be an important factor to take into consideration when designing future studies.

The approach of Mann et al. proved a successful discovery method for known and novel co-drivers of BRAF-activated melanomas and demonstrated the utility of lineage-restricted mutagenesis for cancers with high mutation rates. Notably, the results of this analysis support a model of melanoma progression in which cumulative haploinsufficiencies in TSG allow strong oncogenic drivers to reach their full potential. Davoli et al. (2013) provided evidence suggesting that tumors may rely on many more drivers than had been previously suggested and that these drivers exist on a continuum of phenotypic potency. The power of the SB mutagenesis system lies in its ability to detect drivers at all levels in the well-populated mutational landscape of melanoma.

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high copy [*Sleeping Beauty*] mutagenesis could be an effective method to screen for haploinsufficient TSGs

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