Advantages of whole-genome sequencing for identification of tumor etiology and clinically actionable genomic aberrations: lessons from the Australian Melanoma Genome Project

“These differences have implications for clinical mutation testing and provide a unique insight into the biology of melanoma development.”

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Whole-genome sequencing (WGS) is currently the most comprehensive single assay for surveying the genomic landscape of a patient's tumor genome. The information gained from analyzing millions of nucleotide sequences can inform clinicians on the etiology of the tumor, identify the presence of mutations in critical driver genes of oncogenesis, detect potential therapeutic targets and predict prognosis. We have recently published the largest collection of melanoma whole-genome sequences to date, as part of the Australian Melanoma Genome Project (AMGP) [1]. The project included a wide range of melanoma histosubtypes, which revealed dramatic differences in the

types of mutations that occur in cutaneous melanoma compared with melanomas that arise in acral and mucosal surfaces of the body. These differences have implications for clinical mutation testing and provide a unique insight into the biology of melanoma development.

Expanding the categories of genomic alterations we can detect & treat

The AMGP revealed that melanomas arising in cutaneous sites had on average 18-times more single nucleotide variants than acral and mucosal melanomas [1]. However, acral and mucosal melanomas are dominated by large structural

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rearrangements of chromosomes (three-times more than cutaneous melanomas), such as large-scale gene deletions, insertions, inversions and fusions. These structural variants (SVs) are not often tested for in routine clinical-targeted panel genotyping and the most comprehensive method for detecting them is with WGS. The AMGP found that SVs contribute a significant proportion of the mutations to known melanoma genes, which includes the *NF1* gene that makes up the third most common melanoma genotype [2]. The study found that 19% (6/32) of the *NF1* mutant melanomas were altered via structural rearrangements without an accompanying point mutation, with another three cases possessing both a structural variant and a point mutation. Therefore, testing for single nucleotide variants alone with targeted mutation testing panels that do not assess for structural variants will result in the under-detection of the clinically actionable *NF1* mutated cases and this represents a significant proportion of patients. Therefore, mutation testing should include SV analysis, either on a global scale with WGS or built into targeted sequencing panels for SV-sensitive genes such as *NF1*. This will expand the proportion of patients that can be triaged into appropriate treatment strategies for the administration of targeted small molecule inhibitors.

Noncoding mutations

Approximately 1–2% of the human genome is transcribed into protein and while the other 98% is not protein coding (noncoding regions); it contains regulatory regions of protein-coding genes and also encodes noncoding RNAs that regulate gene or protein expression [3]. Mutations in nonprotein coding regions can affect gene expression by altering the binding sites of positive and negative regulators of the particular gene [4]. The AMGP provides the first detailed characterization of noncoding mutations in a range of melanoma subtypes. The most common of all mutations in cutaneous melanoma occurs in the noncoding promoter region of the *TERT* gene, which has been shown to maintain telomere ends and enable cancers to evade apoptosis [5]. Our study found that 86% of cutaneous melanomas harbored a *TERT* promoter mutation, which is in line with prior studies [6]. However, we found only 11% of acral and mucosal melanomas contained *TERT* mutations, again pointing to some major differences in the biology of the melanoma histotypes. Interestingly, we found no significant

correlation between mutations to the *TERT* promoter or other known mechanisms of telomere elongation and increased length of melanoma cell telomeres. We also discovered many novel high-frequency recurrent mutations in the non-coding regions, though relatively few of these mutations result in changes in gene expression and their function in melanoma remains uncertain [1]. Nevertheless, this is an exciting area of research, and further work on larger number of samples is needed to better understand the role of noncoding mutations in melanoma biology and their possible implications for therapy.

What are the carcinogens driving melanoma: mutational signatures give key insights

The mutagenic processes that drive the development of a tumor leave a unique mutational signature in the nucleotide sequences of the tumor genome [7]. Ground-breaking work has led to the deciphering of some of these mutational signatures and linked them to their causative mutagens [8]. These include but are not limited to exposure to ultraviolet radiation (UVR), smoking, RNA editing dysfunction, altered DNA repair and many other signatures for which the causative mutagen remains unknown. WGS enabled us to comprehensively catalog the mutational processes impacting a diverse range of melanoma subtypes. As expected, our study demonstrated that exposure to UVR was the dominant driver of accumulation of mutations in cutaneous melanomas. However, the use of WGS yielded unprecedented resolution in mapping these mutations and enabled us to further separate the UVR signatures into three novel subgroups. These subgroups differed in the type of lesion UVR induced in the DNA and may relate back to types and patterns of UVR exposure and indirect UVR damage. Understanding these signatures may help with melanoma prevention strategies, as we link the mutations to specific patterns of UVR exposure. Conversely, acral and mucosal melanomas displayed non-UVR-related mutational signatures, similar to those seen in breast cancer and medulloblastoma, which relate to dysfunctional RNA editing, age and many other signatures of unknown causation [1,7].

A practical use of mutational signatures was recently demonstrated in a study by McGranahan and colleagues [9]. The group performed exome sequencing on a large cohort

of early stage non-small-cell lung cancers and found that patients who had a history of smoking, unsurprisingly had a tumor genome dominated by smoking-induced mutations. The surprise was an outlier patient that never smoked, whose mutational profile was similar to that of the smoking patient's (high number of mutations with nucleotide transition consistent with smoking). With further investigations into the clinical information of the patient, the group found that the patient had worked in the petrochemical industry and was a road worker for many years and had subsequently been exposed to bisphenol A, hydrocarbons and many other toxic chemicals. Therefore, the analysis of genome-sequencing data was able to direct the team to the mutagen exposure that can be attributed to the development of a patient's tumor. The implications of which could be that the signatures from many other patients with different cancers could be traced back to causative mutagens, thereby leading to a greater understanding of the causes of cancer and help guide appropriate preventative measures.

Clinical applications

Our recent study has two major implications to the clinical care of melanoma patients. First, major tumor suppressor genes relevant to melanoma, *NF1*, *CDK2NA* and *PTEN* are particularly susceptible to structural rearrangements that confer a loss of function to these genes and mostly occur in

acral and mucosal melanomas. Therefore, clinical mutation testing platforms need to account for the high rate of structural rearrangements in these genes to obtain accurate molecular workups for melanoma patients. Second, mutational signatures or molecular mutagenic fingerprints in the tumor genomes can be used to identify the mutagenic driver of cancer development and progression of an individual patient's disease [1]. This information could be used to inform melanoma prevention strategies and to identify patients who are particularly susceptible to certain mutagens.

Conclusion

We are only scratching the surface of the utility of genome-sequencing technologies and while many cost and infrastructure hurdles remain, the promise that the technologies offer in the understanding of tumor biology and implications for treatment mean that the technologies will grow in use and practicality.

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