The landscape of driver mutations in melanoma: Creating a framework for targeted and immune therapy

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Dr. Watson, in his presentation at the Banff meeting, described recent research in melanoma genetics and provided an overview of some of the Canadian collaborative initiatives underway.

In 2012, the reported survival outcomes of patients with melanoma treated with the checkpoint inhibitor, ipilimumab, and monotherapy with BRAF inhibitor-targeted therapy, vemurafenib, set the goals for the research community. With ipilimumab, most patients had no benefit, but about 20% showed durable long-term response. With vemurafenib, patients with BRAF mutation showed good initial response, however most eventually developed resistance.[1] The research community's goal over the past 7 years, said Dr. Watson, has been "to increase these survival curves and find ways to develop new therapeutic strategies or combination therapies in order to develop long-term durable responses in a majority of patients."

LANDSCAPE OF DRIVER MUTATIONS

Melanoma is caused by hotspot mutations in BRAF and NRAS genes in approximately 50% and 20% of patients, respectively. Efforts to identify the genetic driving events in BRAF/NRAS wild-type melanomas became possible with advances in next-generation sequencing technology. Researchers at the Broad Institute in Cambridge, Massachusetts, sequenced mutations in a cohort of 121 melanoma tumour/normal pairs, found that a very large number of genes mutated, and used statistical tools to determine the gene-specific background mutation burden that would then help identify driver mutations. The study[2] identified the UV exposure signature mutations that were driving the cancer. These included a hotspot of oncogenic mutations in the Rho guanosine triphosphatase (GTPase), RAC1, in approximately 5%–10% of patients from sun-exposed skin, and frequent loss-of-function (LoF) mutations in genes involved in chromatin remodelling. Subsequent studies validated RAC1 mutations at an incidence of about 3.5% in a larger number of samples.[3]

"We then looked at the genomic copy number data and found that RAC1 was significantly amplified in about 20% of samples," said Dr. Watson, "pointing to the role of RAC1 P29S in regulating a number of signalling pathways that drive mutation and tumour growth."[4] Recent research showing that these RAC1 mutants may be sensitive to p21-activated protein kinase 1 (PAK1) inhibitors, while requiring confirmation, provides a potential opportunity to develop new therapeutic strategies to treat melanoma.[5]

"Our next-generation sequencing studies helped delineate driver mutations that have these UV signature mutations and C to T transitions," says Dr. Watson. They did not identify the next BRAF mutation, however. They then carried out comprehensive molecular profiling on 331 cutaneous melanoma cases (with Dr. Jeffrey Gershenwald and Dr. Lynda Chin) and identified all the known melanoma driver mutations, including BRAF, NRAS, CDKN2A, TP53 and PTEN,

along with the genes identified in their first study—PPP6C, ARID2, MAP2K1 and RAC1— as well as other genes found to be significantly mutated in melanoma, including IDH1, DDX3X, NF1 and RB1. Looking at the landscape of somatic mutations in these samples, they most frequently found, beyond the BRAF and NRAS mutations, an accumulation of NF1 LoF mutations.

NF1 LoF mutations are found in over 50% of patients who do not have a BRAF or NRAS mutation. NF1 is a well-established tumour suppressor gene that encodes for neurofibromin, a protein that negatively regulates RAS signalling. Germline mutations in NF1 are known to lead to neurofibromatosis type 1, a multisystem genetic disorder that includes the clinical appearance of melanocyte defects. Somatic mutations in NF1 occur in leukemia, breast, brain, ovarian and lung cancers. These findings extended earlier evidence that BRAF and NRAS mutations were mutually exclusive, while adding that the BRAF hotspot and NF1 LoF mutations were in fact anticorrelated.

The Cancer Genome Atlas (TCGA) analysis working group proposed that melanoma could be categorized into 4 genomic subtypes: hotspot BRAF (generally younger patients) and hotspot RAS (N/H/K) mutations were known, "and we proposed a new melanoma subtype, NF1," recounted Dr. Watson, "which possessed the loss-of-function mutation. These patients are generally older and have a higher mutation burden. And there was this fourth subtype, which we called triple wild-type melanomas." Looking then at what was driving these last melanomas, Dr. Watson and colleagues observed that these melanomas have amplifications in oncogenes including KIT and other receptor tyrosine kinases (RTKs), and MDM2, CDK4, CCND1 and TERT. The copy number alterations and amplifications seen in these triple wild-type melanomas are consistent with work from Boris Bastian published in 2005 of cyclin D1 and CDK4 being amplified in this subset of melanoma.[6] Integrative analysis of TCGA data allowed the researchers to take an unbiased look at gene expression patterns within melanoma: "None correlated with the hotspot mutations in BRAF, NRAS, NF1 or the triple wild-type subtypes," said Dr. Watson. "What they were indicative of was immune infiltration." Richard Scolyer's group in Sydney, Australia, carried out pathology analysis of all the samples and observed that patients with increased lymphocytic infiltration had, as expected, longer survival and clustered with an immune expression mRNA signature.

CLINICAL SIGNIFICANCE

In the arena of targeted therapy, BRAF and MEK inhibitors are currently being used for BRAF hotspot-mutated patients. The NRAS mutant patients are getting variable results with the MEK inhibitors. Similarly, the NF1 mutant melanomas are also having this variable degree of response to MEK inhibitors, at least in 3 preclinical studies published around the time of the TCGA publication.[7,8,9] With NF1 loss, the MAP kinase pathway would be activated, and at least some melanomas responsive to MEK inhibitors would be expected. A study done at Yale University by Michael Krauthammer and Ruth Halaban showed that there is a fraction of melanoma samples responsive to MEK inhibitors.[10]

To understand response to targeted therapy, it is important to look at the co-occurrence of BRAF, NRAS and NF1 mutations. While NF1 mutations are anticorrelated with the hotspot BRAF mutations, the NF1 immune subtypes also have an enrichment of these BRAF non-V600E mutations. The non-V600E mutant signals that seem to co-occur with NF1 mutations are RAS-dependent ones. They require either a loss of NF1, a co-occurring NRAS mutation, or amplification of an RTK. In melanoma, it is usually a LoF mutation in NF1.

COMBINING TARGETED THERAPIES

Dr. Watson's group has a Melanoma Research Alliance grant to try to tease apart the interaction of all these different mutations and find ways to best combine targeted therapies with various BRAF mutants. This is especially important to figure out the best way to combine targeted therapy with immunotherapy for NF1-mutant patients, given that a number of recently published studies have demonstrated that NF1-mutant melanomas respond better to anti–PD-1 therapy. A study from Massachusetts General Hospital and Vanderbilt looked at 32 responders and 33 nonresponders to anti–PD-1 therapy[11], finding that among NF1-mutant patients, 16 responded (50%), compared to 7 who did not (21%). This was the only significantly mutated gene found to correlate with response to anti–PD-1. In early 2018, Dr. Georgina Long and Dr. Antoni Ribas published an exciting study showing that in desmoplastic melanoma, where the NF1 subtype is predominant, around 70% of patients had an objective response and, of the 17 patients they were able to sequence, 80% had a LoF mutation.[12]

Melanoma has a higher mutation burden than other cancers, and many more melanomas will need to be sequenced to identify a fuller range of driver mutations. In a pangenomic analysis of all published melanoma exome sequencing studies, Dr. Watson's group is finding additional driver mutations in this cancer that might provide opportunities for developing new therapies. Some are low-frequency significantly mutated genes, while others are high-frequency significantly mutated genes involved in epigenetic regulation.

Dr. Watson and colleagues are now carrying out preclinical studies to identify new therapeutic strategies and have started genomic initiatives in Canada to improve identification and selection of responding and nonresponding patients to melanoma therapy. They are interested in identifying on-treatment biomarkers and BRAF and MEK inhibitors predicting future response to immune therapy, as well as molecular features indicating patient response to, and toxicity of, anti–CTLA-4, anti–PD-1, and combination therapy.

COLLABORATIVE APPROACH

Intrinsic, extrinsic and circulating tumour factors need to be analyzed in parallel to determine and compare the best

biomarkers. Dr. Watson and others are working to develop a database or "data commons" with these molecular platforms of patients treated with targeted and immune therapy. They have established the Montreal Melanoma Research Network and are looking to expand this into a Canada-wide melanoma collaboration. Efforts for melanoma biobanking and clinical data collection have been started in Montreal-area hospitals, bringing together expertise with up-to-date molecular platforms to develop a collaborative framework to perform cancer therapy-responsive integrative profiling (cTRIP). This includes immunomonitoring and microbiome analysis at the Centre hospitalier de l'Université de Montréal; tumour antigens and proteogenomics at the Université de Montréal; proteomics at the Jewish General Hospital; T-cell receptor (TCR) sequencing at Hôpital Maisonneuve-Rosemont; genomics and mass cytometry (CyTOF) metabolomics at the Goodman Cancer Research Centre; and circulating tumour cell (CTC) collection at McGill University Health Centre.

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