It's been a burning question in melanoma research: Tumor cells are full of ultraviolet (UV)-induced genetic damage caused by sunlight exposure, but which mutations drive this cancer?

None have been conclusively tied to melanoma. The sheer abundance of these passenger mutations has obscured the search for genetic driver mutations that actually matter in melanoma development and progression.

By creating a method to spot the drivers in a sea of passengers, scientists at the Broad Institute of MIT and Harvard, the Dana-Farber Cancer Institute and The University of Texas MD Anderson Cancer Center have identified six genes with driving mutations in melanoma, three of which have recurrent 'hotspot' mutations as a result of damage inflicted by UV light. Their findings are reported in the July 20 issue of the journal *Cell*.

"Those three mutations are the first 'smoking gun' genomic evidence directly linking damage from UV light to melanoma," said co-senior author Lynda Chin, M.D., Professor and Chair of MD Anderson's Department of Genomic Medicine. "Until now, that link has been based on epidemiological evidence and experimental data."

"This study also is exciting because many of the recent large-scale genomic studies have not discovered new cancer genes with recurrent hot-spot mutations, a pattern strongly indicative of biological importance," said Chin, who also is scientific director of MD Anderson's Institute for Applied Cancer Science.

The six new melanoma genes identified by the team are all significantly mutated and provide potential targets for new treatments.

Puzzle has thousands of potential pieces, but only requires a few dozen

A number of important mutations had previously been identified as melanoma drivers. These include BRAF (V600) mutations, present in half of all melanomas, and NRAS (Q61) mutations. However, the vast majority of these mutations do not appear to be caused by direct damage from UV light exposure.

Those known mutations are important, but don't tell the whole story. Melanoma, the authors note, has higher genetic mutation rates than most other types of solid tumors. The majority are attributable to passenger mutations caused by UV light damage resulting in a DNA alteration called a cytidine (C) to thymidine (T) transition.

Chin together with Levi A. Garraway M.D, Ph.D., associate professor at Dana-Farber Cancer Institute and Harvard Medical School and senior associate member at the Broad Institute, sequenced the exons—active portions of DNA involved in protein synthesis—in 121 melanoma samples paired with normal DNA and found 86,813 coding mutations. The resulting mutation rate was higher than that ever reported in any other tumor type.

Among the most frequently mutated genes, 85% of the active coding mutations resulted from C to T transitions caused by UV light exposure.

Statistical approaches to identify driver mutations have often assumed that the baseline mutation rate is uniform across the genome. The abundance of UV-induced passenger mutations that vary in frequency confounds this assumption in melanoma, the researchers report.

"When a gene is found to be repeatedly mutated, we naturally assume that it must be important to the cancer," said Garraway, who is co-senior author with Chin on the study. "However, melanoma can fool us—in that cancer, the very high mutation rate means that many genes can be recurrently mutated purely by chance. We needed a solution to this problem."

To counter this effect, the researchers turned to parts of the genome that don't code for proteins, called introns, and other inactive DNA segments that flank exons. By comparing the frequency of mutations in the inactive segments to the frequency of mutations in the exons, the researchers built a framework for assessing the statistical significance of functional mutations.

Approach identifies six known cancer genes, six new ones

The analysis identified functional mutations in the well-known cancer genes BRAF, NRAS, PTEN, TP53, CDKN2A and MAP2K1.

It also uncovered five new genes, RAC1, PPP6C, STK19, SNX31, and TACC1. Most are associated with molecular pathways involved in cancer but had not been previously recognized as significantly mutated in melanoma. Their presence in the tumor samples ranged from 3% to 9%.

The sixth new gene tied to melanoma was ARID2, an apparent tumor-suppressor gene possessing a significant number of loss-of-function mutations found in 7% of patient samples.

"Six new melanoma genes have been picked out from thousands of mutated genes," said Eran Hodis, co-lead author who is a computational biologist in the Garraway lab at the Broad Institute and an M.D.-Ph.D. student at Harvard and MIT. "The same approach may bring clarity to genome sequencing studies of other cancers plagued by high passenger mutation rates, for example lung cancer."

UV damage causes 46% of driver mutations

The team then cross-referenced their findings with a database of recurrent mutations called COSMIC and gained new insights in the frequency and characteristics of driver mutations, old and newly discovered, in 21 genes.

Out of 262 driver mutations in the 21 genes, 46% were caused by UV-induced damage. The well-known tumor-suppressing gene TP53 had the greatest number of UV-caused mutations. Other tumor-suppressors also had loss-of-function mutations and all of the newly identified genes had a high percentage of mutations caused by UV damage.

Most exciting, three of the discovered genes possessed 'hotspot' mutations found in the exact same position in multiple patients providing another line of evidence indicating these mutations contribute to melanoma.

"We have now discovered the third most common hotspot mutation found in melanoma is present in a gene called RAC1, and unlike BRAF and NRAS mutations, this activating mutation is attributable solely to characteristic damage inflicted by sunlight exposure" said Ian R. Watson, Ph.D., co-lead author of the study and postdoctoral fellow in the Chin lab at MD Anderson.

New insights provide opportunity to better understand, treat melanoma

Much work remains following the most comprehensive analysis of the genetics of melanoma, the authors noted. If diagnosed early, melanoma is highly curable, but in its metastatic stage is lethal. Determining the role these mutated genes play in biological processes important for melanoma progression and metastasis provides a new avenue of investigation into the molecular basis of this disease.

With the advent of the BRAF inhibitor vemurafenib, melanoma has emerged as the latest success story for genomics-guided targeted therapy in treatment of patients with metastatic disease. However, melanoma eventually resists this therapy and effective treatment options for patients that do not possess a BRAF (V600) mutation are limited.

Determining whether these newly discovered genes are amenable to targeted therapy, or whether their mutations predict sensitivity to currently available drugs, Chin said, will be an important next step in translating these findings into the clinic.

Source: University of Texas M. D. Anderson Cancer Center