Deeper look at biopsy exposes mutation ready to ambush drug combination

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A powerful resistance mutation that appeared to emerge in melanoma after a patient received a targeted therapy combination, instead was lurking in the tumor all along, primed to thwart treatment before it began, researchers at The University of Texas MD Anderson Cancer Center report online at *Cancer Discovery*.

Researchers analyzed a series of biopsies taken before and during treatment to ferret out the pre-existing mutation and then developed a potential way to target its troublesome abilities.

The team, led by Lawrence Kwong, Ph.D., assistant professor of Translational Molecular Pathology, set out to find resistance mechanisms that arise against a combination of MEK and CDK4 inhibitors to treat melanoma that has a mutation in the NRAS gene.

The mutation, to a gene called PIK3CA, appeared initially to be an acquired resistance variation that arose after treatment. By re-analyzing the pretreatment biopsy, Kwong and colleagues were able to establish that it was rare but present from the start, hiding on one side of the tumor.

PIK3CA variant started rare, expanded rapidly

"Our study is the first to measure multiple regions in pre-treatment tumor biopsies at high resolution and then track the resistant mutation

over years of treatment through six biopsies," Kwong said. "We are able to say that this mutation started out rare and then rapidly expanded as the MEK/CDK4 inhibitors killed off a large number of non-resistant cells."

This finding helps establish that such pre-existing mutations can lurk in a patient's tumor at 10 times the rarity than previously appreciated and still cause rapid drug resistance, raising the possibility that even more rare mutations exist in other patients, below the detection rate of current technology.

"Right now, when we detect a resistance mutation after treatment, we often don't know whether it came out of nowhere as a new mutation or was pre-existing but undetected in the original tumor," Kwong said.

Understanding the difference could guide treatment to make it more effective, earlier, Kwong notes, and identifying rare mutations that are geographically isolated on a tumor will require improving our approach to analyzing biopsies.

NRAS mutations occur in 15-20 percent of melanomas, and the MEK/CDK4 combination is often effective initially against these tumors, but resistance arises.

Initial response, then swift progression

A 59-year-old woman with stage III malignant melanoma was found to have an NRAS mutation in her tumor. She was enrolled in a clinical trial combining a MEK and a CDK4 inhibitor. After an initial partial response of a 39 percent reduction in tumor burden, resistance to the treatment arose swiftly and the disease progressed and spread.

Whole exome sequencing of the resistant tumor after treatment revealed a mutation to PIK3CA known to promote tumor growth. Since the

mutation was detected only 16 days after treatment began, Kwong and colleagues decided to re-examine the pretreatment biopsy, which sampled a single region of the tumor and had not found a PI3KCA mutation.

By examining seven regions of the biopsy sample using an amplification method developed by co-author David Zhang, Ph.D., assistant professor of Bioengineering at Rice University, the team found PIK3CA mutations in three regions. The pre-existing mutation was both rare and geographically dispersed in the tumor, making it hard to detect by sampling a single region.

Their findings suggest multi-region sampling would expose pre-existing resistant cells, an approach that would not be cost-effective at present, Kwong said, but is likely to become more practical as technology develops.

The PIK3CA mutation could also be detected by isolating circulating cell-free DNA in the blood after resistance developed, making it a potential target for liquid biopsies that are under development.

S6 provides a common target

Simply adding a PIK3CA inhibitor to the MEK/CDK4 combination would likely be too toxic, so the team analyzed 300 proteins to find targets that might be present in more than one of the three pathways.

They found a protein called S6 to be the only spot where all three of these cancer-promoting pathways meet. Treating mice with an S6 inhibitor re-sensitized them to treatment with the MEK/CDK4 combination, restoring the drugs' ability to shrink the PIK3CA mutation-bearing melanomas.

Kwong said an optimized human version of the S6 inhibitor in mice has not yet been developed, but their findings point to a possible target for human drug development.

"One of the main questions in cancer drug resistance is how often it comes from a pre-existing or a completely new mutation" said Gabriele Romano, Ph.D., a postdoctoral fellow in Translational Molecular Pathology and the study's first author. "Our study helps define some of the parameters and tools that will be needed to answer this tricky question."

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