Scientists discover innate tumor suppression mechanism

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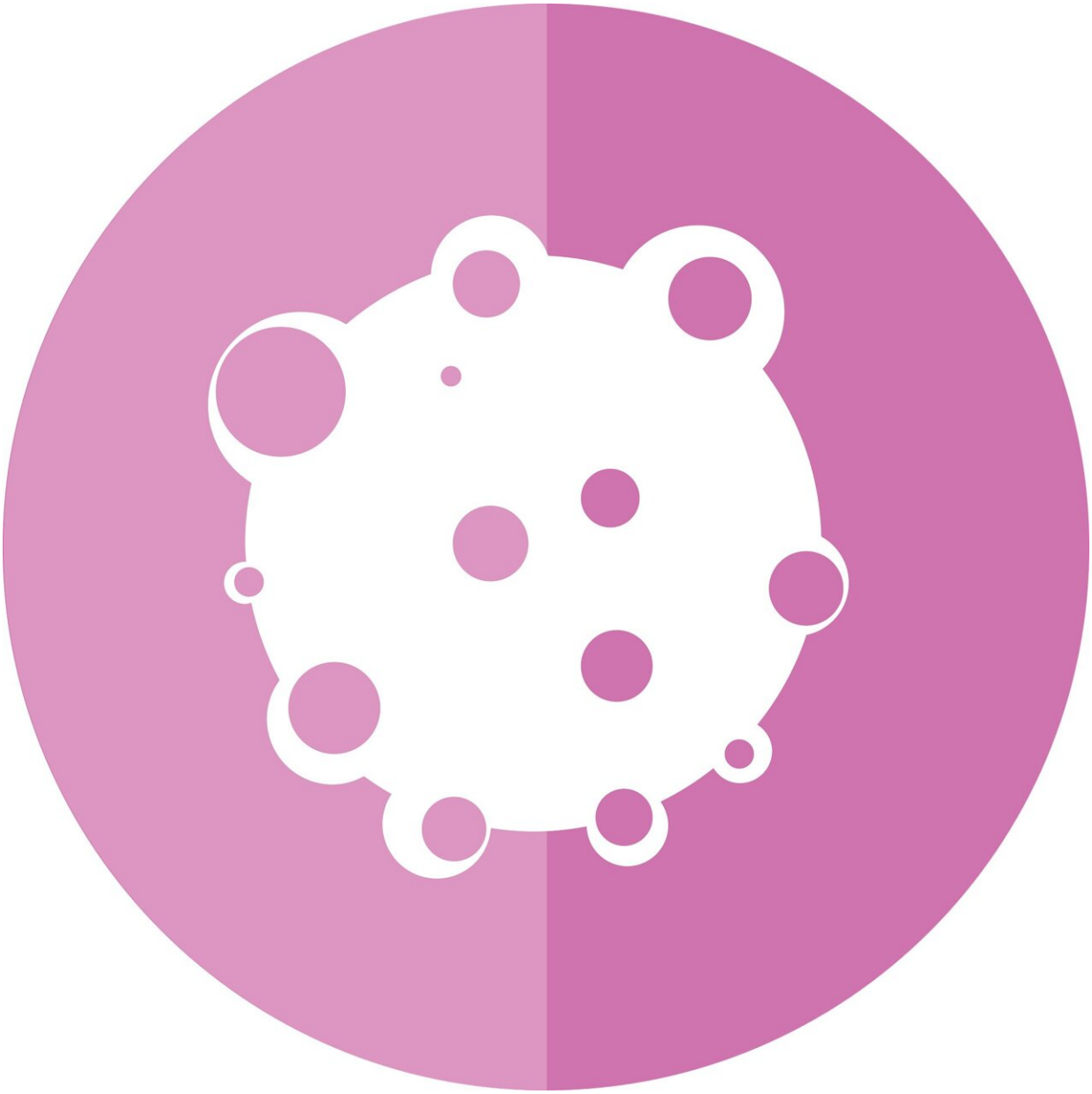

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The p53 gene is one of the most important in the human genome: the only role of the p53 protein that this gene encodes is to sense when a tumor is forming and to kill it. While the gene was discovered more than four decades ago, researchers have so far been unsuccessful at determining exactly how it works. Now, in a recent study published in *Cancer Discovery*, researchers at The Wistar Institute have uncovered a key mechanism as to how p53 suppresses tumors.

By using a genetic variant of p53 and comparing what that variant failed to accomplish with what the healthy "wild type" p53 gene could do, the researchers discovered the mechanism by which p53 triggers immune function that, in turn, kills the tumor.

"The paradigm shift is that, instead of asking 'What does p53 do' we were able to use a lesser-functioning but cancer-predisposing genetic variant in African Americans to tell us 'What does p53 not do when it doesn't suppress cancer?'" said Maureen E. Murphy, Ph.D., senior author on the paper and deputy director of the Ellen and Ronald Caplan Cancer Center and Ira Brind Professor and program leader of the Molecular & Cellular Oncogenesis Program at The Wistar Institute.

Four and a half million people in the United States possess inherited, or germline, mutations in p53, which increases their risk of cancer. A small subset of these individuals have a mutation that leads to Li Fraumeni Syndrome, which results in their developing multiple tumors every few years, starting in childhood.

Others with different p53 mutations possess what are called hypomorphs: a gene variant having a similar but weaker effect than the

corresponding normal, or wild-type, gene. These people also develop cancer, but theirs is less aggressive, and they develop it later in life.

Murphy and her team decided to learn how p53 suppresses tumors by exploring how one particular hypomorph fails to suppress them. The researchers chose an African-specific variant called Y107H due to the fact that African Americans have the largest cancer burden of any ethnic group in the world.

Their first hypothesis was that they could use the hypomorph to find which "downstream" genes—which p53 would ordinarily turn on—are critical for suppressing tumors. Their second hypothesis was that they could then screen for drugs that would kill the hypomorph tumors: Murphy's group was able to accomplish both goals.

The researchers began by using CRISPR engineering to make a mouse model of their African-specific hypomorph Y107H. As expected, the mice with Y107H developed many forms of cancer and, as with humans who possess this variant, they started developing cancer in "middle age" (i.e., after 12-14 months of an average two-year lifespan).

Next, the researchers created tumor cell lines with their Y107H hypomorph, as well as cell lines with a hypomorph found in Ashkenazi Jewish populations, called G334R. They then compared which genes were turned on by normal, or wild type, p53 (to suppress the tumor) but not turned on by the two hypomorphs (which failed to suppress the tumor). The gene that met these conditions was PADI4. To confirm, they checked ten other hypomorphs—none of those variants turned on PADI4, either.

"It's as though this was the key p53 target gene that, every time you have a genetic variant that predisposes you to cancer, it cannot turn on this gene," said Murphy. She added that it makes sense that PADI4 would be

implicated, because this gene helps the immune system recognize tumors. It does this by modifying components of tumor proteins so that they become citrulline, which is a non-natural amino acid. When the immune system recognizes citrulline as a foreign body, it attacks.

"Essentially, when a tumor cell goes from one cell to two and it's not supposed to, p53 is alarmed, it turns on PADI4, and PADI4 says, 'Immune system, you better come get me,'" said Murphy.

The final stages of Murphy's research went beyond foundational research and looked toward helping cancer patients. First, the researchers used Wistar's Molecular Screening and Protein Expression facility to identify drugs that would be effective against tumors with the Y107H hypomorph while sparing tumors with wild-type p53.

Then, they looked for a way to predict which patients would respond to immunotherapy and which would not. Ordinarily, in order to do this, they would need many more human tissue samples from African Americans than they had. So instead, they turned to machine learning.

"Enter Noam Auslander, Ph.D., who is a brilliant machine learning artificial intelligence person here at Wistar," said Murphy. "She said, 'Let me find the genes that p53 and PADI4 control together using bioinformatic approaches and create a gene signature.'"

To do this, Auslander analyzed 60,000 tumors in the TCGA database and identified five genes that were coregulated together by wild type p53 and PADI4 and that the Y107H hypomorph couldn't turn on. Upon further analysis, she found that this five-gene signature predicted cancer survival, immune infiltration into the tumor, and who would respond to immunotherapy.

Murphy believes that identifying this gene signature through machine

learning was what pushed her team's paper from a scientific breakthrough to a medical game-changer. "We've not only said we have an important p53 target gene, but we also have an important five-gene signature that will actually tell us who will respond to immunotherapy and who won't, and p53 is at the core of this signature."

She also believes that this research could only have been performed at an institution like Wistar, because collaboration was so crucial. "If you look at the authors on this, I have immunologists who did the immunology; I have machine learning people who did the bioinformatics; and I have drug screening people who did the compound screens," said Murphy. "Wistar is just a thrilling place where everyone here is saying, 'Here's how I can help your research.' It makes all the difference."

More information: Alexandra Indeglia et al, An African-specific variant of TP53 reveals PADI4 as a regulator of p53-mediated tumor suppression, *Cancer Discovery* (2023). DOI: 10.1158/2159-8290.CD-22-1315

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