CRISPR screen reveals loss of genes in interferon gamma receptor signaling pathway

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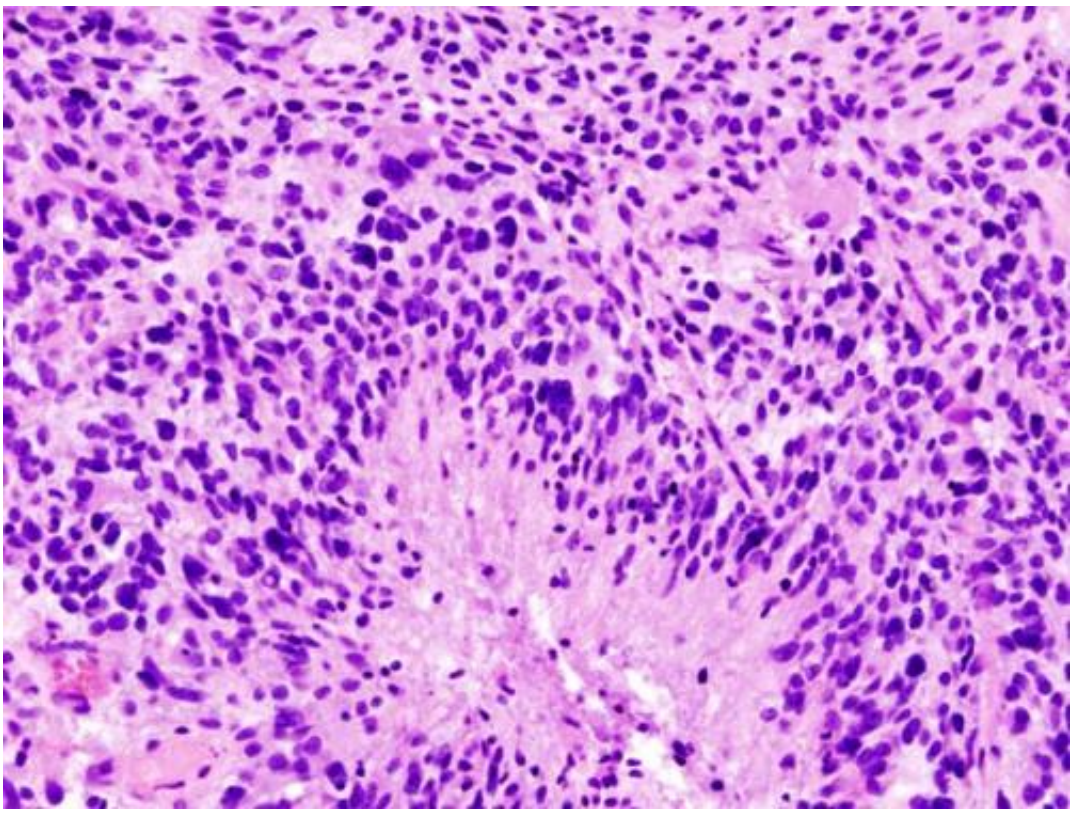

Glioblastoma (histology slide). Credit: Wikipedia/CC BY-SA 3.0

Researchers at Massachusetts General Hospital (MGH) have discovered that the interferon gamma receptor (IFNgR) signaling pathway is critical for susceptibility of glioblastoma tumors to killing by CAR T-cell

immunotherapy. The same phenomenon was observed in other solid tumors. This discovery may partly explain why liquid and solid tumors respond very differently to CAR T-cell treatment. The research is published in *Nature*.

A chimeric antigen receptor (CAR) is any synthetic molecule that specifically commands the T cells of the immune system to identify and stick onto a target, or antigen. CARs recognize targets that are on the surface of tumor cells. While CAR therapy has had a transformative impact on the treatment of hematologic cancers like leukemia and lymphoma, it has not this translated into similar success in solid tumors.

To identify the resistance pathways in solid tumors, researchers led by Marcela Maus, MD, Ph.D., director of the Cellular Immunotherapy Program at the Mass General Hospital Cancer Center developed a genome-wide CRISPR knockout screen in glioblastoma.

"With a CRISPR screen we were able to interrogate the entire genome in a pooled format in a completely unbiased manner, instead of looking for one or two genes of interest at a time," explains first author Rebecca Larson, Ph.D. This allowed the researchers to see which genes are lost and determine the mechanisms of resistance that solid tumors use to evade CAR T-cell therapy. In this study, they applied selective pressure with a CAR to each barcoded cell in the screen. "We then sequenced the cells and could see which tumor cells were alive afterwards telling us which genes were knocked out."

When Larson and colleagues applied the screen in multiple glioblastoma cell lines, including several cell lines derived from patients, they unexpectedly found that loss of genes in the interferon gamma signaling pathway rendered them resistant to CAR T-cell killing. "That means those interferon gamma-related genes are necessary for the tumor to die in the face of a CAR, something we had not known before and that we

did not expect," adds Larson.

This same resistance pattern was also found in vivo in knock-out mice models. Further study in other solid tumor types, including pancreatic, ovarian, and lung cell lines, showed the same: resistance to CAR T-cell therapy resulted from loss of interferon gamma pathway genes.

"We found that CAR T-cells did not bind to glioblastoma cells lacking interferon gamma signaling," Larson explained, adding that while interferon gamma does not kill the cancer directly, it makes tumor cells stickier. "That way, the CAR T-cell can bind to it better and eliminate the cancer cell."

Conversely, the researchers observed that the interferon gamma pathway did not have a role on the sensitivity of leukemia, lymphoma, or multiple myeloma to CAR T-cell therapy. "The fact that we can see how solid and liquid tumors are responding to CAR T-cell therapy in different ways is very informative for how we design a future therapy."

Moving forward, this discovery gives researchers an opportunity clinically on two fronts, according to Maus. First, enhancing T-cell/tumor cell-binding interactions by targeting the interferon gamma pathway may yield improved responses with CAR T-cell therapy in solid tumors. Second, blocking this pathway in liquid tumors may help reduce the well-known toxicities of CAR T-cell therapies, known as cytokine release syndrome. "Even though CAR T-cell treatment can in some cases be amazingly effective with more than 40% cure rates in some liquid tumors, toxicity is a real concern," she adds. "Tamping down the interferon gamma in these cancers might keep the efficacy but reduce the rollercoaster of toxicity."

More information: Rebecca C. Larson et al, CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours, *Nature*

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