Researchers unlock the door to tumor microenvironment for CAR T cells

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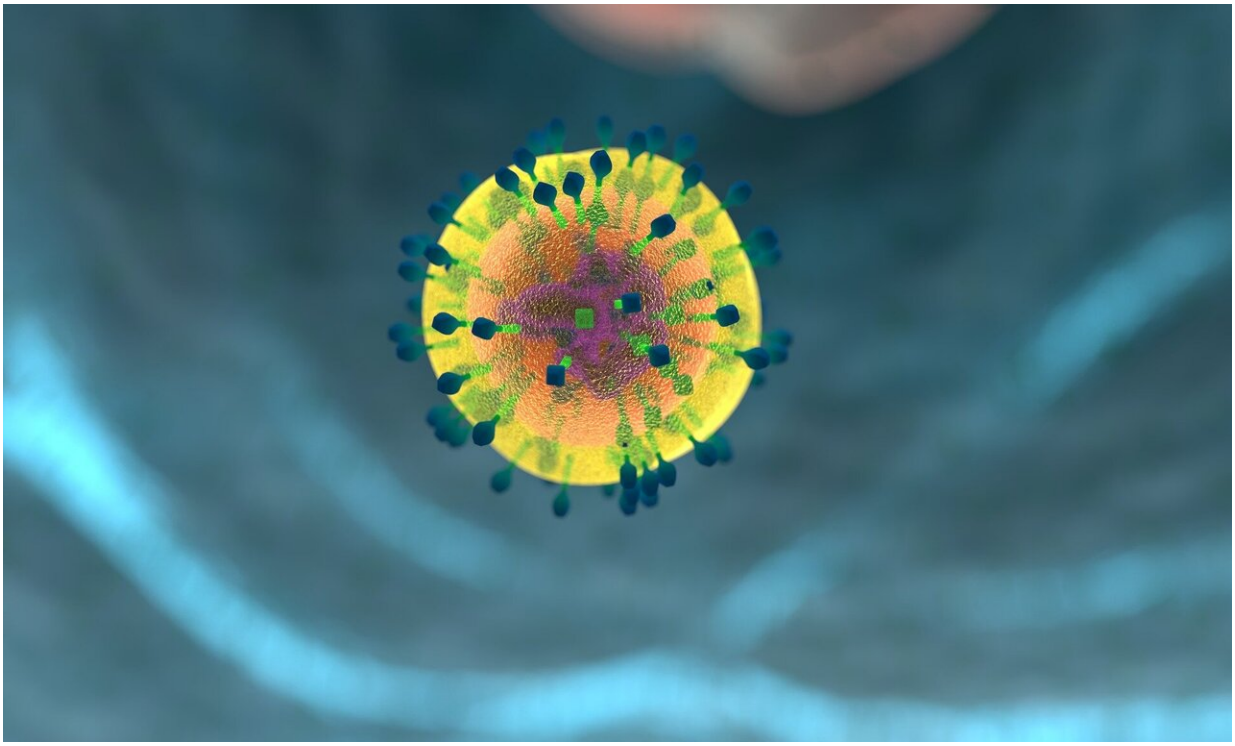

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The labyrinth of jumbled blood vessels in the tumor microenvironment remains one of the toughest blockades for cellular therapies to penetrate and treat solid tumors. Now, in a new study published online today in *Nature Cancer*, Penn Medicine researchers found that combining chimeric antigen receptor (CAR) T cell therapy with a PAK4 inhibitor

drug allowed the engineered cells to punch their way through and attack the tumor, leading to significantly enhanced survival in mice.

The researchers discovered in laboratory experiments that vascularization in solid tumors is driven by the genetic reprogramming of tumor endothelial cells—which line the walls of blood vessels—caused by an enzyme known as PAK4. Knocking out that enzyme reduced abnormal tumor vascularity and improved T cell infiltration and CAR T cell immunotherapies in glioblastoma (GBM) mouse models, the Penn team found. GBM, the most common and aggressive type of brain cancer diagnosed in more than 22,000 Americans every year, is known for its prominent and abnormal vascularity and being immunologically "cold."

"The response in GBM patient from CAR T cell therapies is universally poor because the CAR T cells have a problem getting into the tumor," said senior author Yi Fan, Ph.D., an associate professor of Radiation Oncology in the Perelman School of Medicine at the University of Pennsylvania. "Our study shows that turning off this endothelial cell genetic reprogramming with a PAK4 inhibitor may help open the door to let both T cells and engineered T cells reach the tumor to do their job."

First, the team performed a kinome-wide screening analysis of more than 500 kinases, or enzymes, that regulate blood vessel activation in human endothelial cells from GBM patients. They found that PAK4, which has previously been shown as a driver of growth in solid tumors, was the culprit. Knocking that enzyme out in GBM endothelial cells with a drug, they found, restored expression of adhesion proteins that are important for the recruitment of immune cells and stimulated T cell infiltration into the tumors. Notably, knocking down PAK4 shifted the endothelial cells' morphology, from a spindle-shaped appearance to a characteristic cobblestone in GBM, indicating a less chaotic formation of blood vessels. In other words, it "normalized" the microenvironment.

Next, in a GBM mouse model, they found that inhibiting PAK4 reduced vascular abnormalities, improved T cell infiltration, and inhibited tumor growth in the mice. Approximately 80 percent of PAK4 knockout mice survived for at least 60 days after the experiment was terminated, whereas all wild-type mice died within 40 days after tumor implantation.

Another experiment with a EGFRvIII-directed CAR T cell therapy and a PAK4 inhibitor showed a nearly 80 percent reduction in tumor growth compared to mice that only had CAR T therapy five days after infusion. Notably, nearly 40 percent of the mice in the combination therapy group still survived even when all of the mice in the other groups had died 33 days after tumor implantation.

Targeting PAK4 may provide a unique opportunity to recondition the tumor microenvironment, as well provide a much-needed opportunity to improve T cell-based cancer immunotherapy for solid tumors, the authors said. The findings also support the idea that vessel normalization by PAK4 inhibition can improve drug delivery and reduce oxygen deprivation known as hypoxia, leading to improved tumor responses to targeted therapy, radiation and chemotherapy.

"To our knowledge, we are the first to show how we can reprogram the whole vascular microenvironment with a PAK4 inhibitor and promote cellular therapy," Fan said. "Importantly, this may not only be limited to brain tumors; it could potentially be used for all types, including breast, pancreatic, and others, because vascular abnormality is a common feature for almost every solid tumor."

More information: Targeting PAK4 to reprogram the vascular microenvironment and improve CAR-T immunotherapy for glioblastoma, *Nature Cancer* (2020). DOI: 10.1038/s43018-020-00147-8 , www.nature.com/articles/s43018-020-00147-8

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