Targeting unsuspected protein reverses lymphedema, shows 3D in-vitro model study

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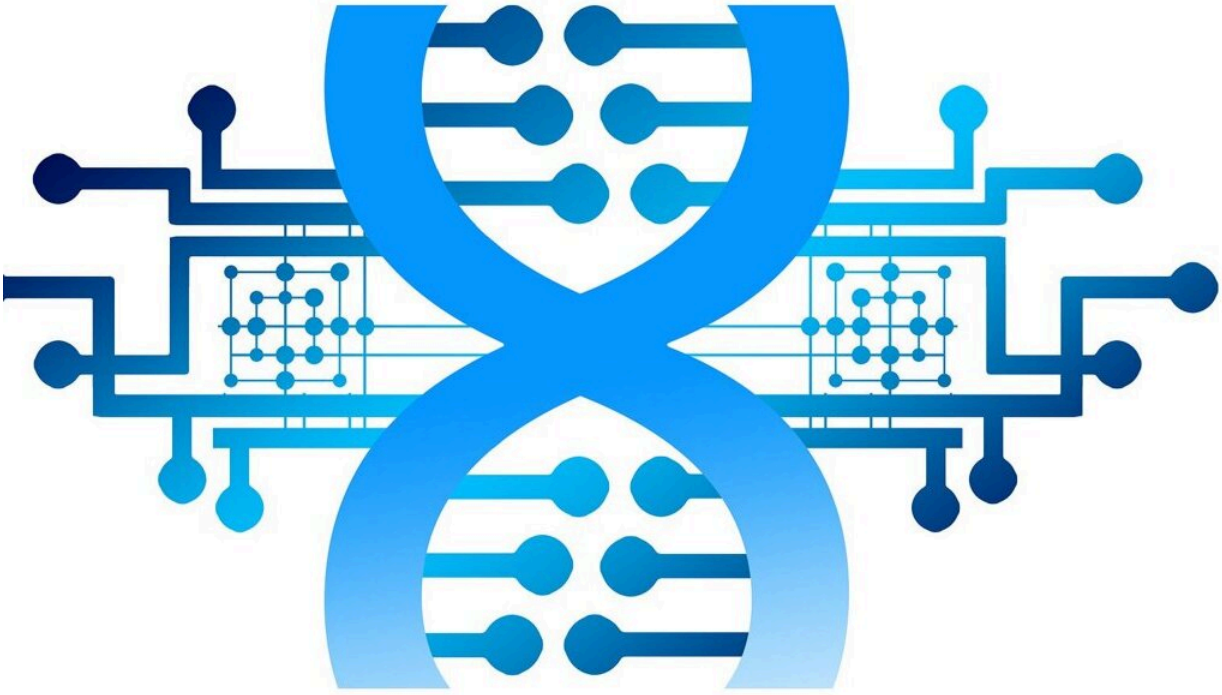

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The human lymphatic system consists of a vast network of vessels that drain protein-rich fluid in tissues and transport it to lymph nodes. When the machinery goes awry and the lymphatic vessels don't drain properly,

the fluid accumulates in the tissues, leading to painful swelling known as lymphedema.

A Cornell-led collaboration built a 3D in-vitro model of a functional human lymphatic vessel that revealed a surprising mechanism that can jam up the necessary drainage: a protein expressed in lymphatic endothelial cells called rho-associated protein kinase 2 (ROCK2).

The researchers demonstrated that by inhibiting ROCK2 they can reverse the effects of lymphedema, creating a potential treatment for a condition that is estimated to affect up to 150 million people worldwide.

The team's paper, "A 3D Biomimetic Model of Lymphatics Reveals Cell-Cell Junction Tightening and Lymphedema Via a Cytokine-Induced ROCK2/JAM-A Complex," published in *Proceedings of the National Academy of Sciences*.

The paper's lead author is Esak (Isaac) Lee, Meinig Family Investigator in the Life Sciences and assistant professor of biomedical engineering at Cornell Engineering.

"Lymphedema has so many patients in the world," Lee said. "Doctors usually suggest you wear a compression garment or do some physical therapy, like massage, to pump out all these fluids from your arms and legs. Unfortunately, there's no FDA-approved drug because we don't understand the mechanism of this disease."

Attempts to locate that mechanism in humans have been thwarted by the fact that the lymphatic system is entangled with the central nervous system, muscle movements, and a raft of other bodily processes.

Working with researchers from Boston University (BU) and Harvard Medical School who were led by co-lead author Christopher Chen of

BU, Lee set out to create an in-vitro model—or "lymphatics-on-a-chip"—that could isolate several biological and biophysical factors, such as inflammatory cytokines, ROCK2 signal and interstitial fluid pressure, while mimicking the drainage.

The transparent, thumb-sized device realistically reproduced the "button-like" lymphatic junction—the borderline between the endothelial cells that line the lymphatic vessels—with two hollow channels in 3D collagen. In the first channel, the team seeded these human cells, which were then pressurized by interstitial fluid loaded in the second channel. High fluid pressure opened the junctions of the engineered lymphatics, and the uptake of fluid closed them. The researchers could then measure how much of the fluid drained through the system.

"Demonstrating this button-like junction is so crucial to recapitulate lymphatic function, which has not been successful in vitro before our study," Lee said. "The most important challenge was to make the right conditions: the size of the vessels, the distance between two channels, growth factors used, and the size of interstitial fluid pressure. All these things are really affecting button junctions, so we wanted to make sure they all are realistically mimicking the human biology."

The researchers introduced inflammatory cytokines that are known to be expressed in lymphedema patients, such as interleukin-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which tighten the lymphatic junctions, leading to fluid buildup and lymphedema.

While these cytokines had been previously known to disrupt blood vessel junctions, the model revealed they were actually tightening the junctions between lymphatic endothelial cells and impeding drainage.

When the researchers inhibited ROCK2, the lymphatic junctions loosened and the blood vessel junctions tightened under inflammation,

so that normal fluid drainage could resume.

"There are upwards of 170 to 180 different pan-ROCK inhibitors, but they generally come with serious side effects, such as hypotension, when these ROCK inhibitors block both ROCK1 and ROCK2, two isoforms of ROCK," Lee said. "The side effects of inhibiting ROCK2, however, are minimal, because ROCK2 is more expressed in lymphatic cells than vascular muscle cells in blood vessels, where ROCK1 is highly expressed. This makes it a strong candidate for therapeutics that target lymphatic disease with less vascular toxicity."

Lee's collaborators reproduced the experiment in mice that lose ROCK2 in their lymphatic cells, which showed a dramatic reduction of edema in the animals' tails.

More information: Esak Lee et al, A 3D biomimetic model of lymphatics reveals cell–cell junction tightening and lymphedema via a cytokine-induced ROCK2/JAM-A complex, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2308941120

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