Research scores advance in manipulating T-cells

October 11 2012

Tew and colleagues have found a way not only to get inside naïve T cells, but to deliver bio-active cargo such as proteins and synthetic molecules across that long-locked cell membrane, by using a new synthetic protein transduction domain that mimics natural ones. Credit: UMass Amherst

(Medical Xpress)—Until recently, medical researchers had little hope of experimentally manipulating naïve T cells to study their crucial roles in immune function, because they were largely impenetrable, says polymer scientist Gregory Tew: "So far off limits we could not readily get inside to investigate their workings."

Now, he and colleagues including immunologist Lisa Minter have found

a way not only to get inside naïve T cells, but to deliver bio-active cargo such as proteins and synthetic molecules across that long-locked cell membrane, by using a new synthetic protein transduction domain (PTD) that mimics natural ones. Tew and colleagues call their new macromolecules "PTD mimics" (PTDMs). They are able to slip through the T cell's membrane and deliver a payload of therapeutic small interfering RNA (siRNA).

The invention is "something like a master key, because we can get into cells without their permission, and into difficult-to-access cell types like human T cells," says Tew. "We think it will lead to new advances in fundamental immunology and it also holds great potential for therapeutic applications in the clinic. We hope every immunologist on the planet hears about this delivery breakthrough, because now they can begin to study T cell function in new ways."

Earlier methods required electroporation or the use of viruses, which either decrease cell viability or pose unacceptable risks to patients in a treatment setting, he adds. Tew and Minter's work appears in the current issue of *Molecular Therapy*.

T lymphocytes are a subset of white blood cells that play a critical role in cell-mediated immunity, fighting against invading infections, cancer and HIV, for example. There are several different types of T cells, so named because they mature in the thymus gland. For years, scientists have wanted to study naïve T cells in particular, those that can respond to pathogens the immune system has not yet seen.

Tew, Minter and colleagues saw an unmet need for non-viral, efficient and easily prepared reagents for use in delivering siRNA into difficult-to-enter cell types such as human T cells. Successfully delivering siRNA into naïve T cells would demonstrate efficient gene knockdown without toxicity and provide a powerful new immunologic tool.

The UMass Amherst team leaders say one of the things they tried to pioneer is being inspired by what nature does, then building synthetic mimics of that. "We felt that if we were not limited to the usual amino acid alphabet of proteins, we could do it better. That turned out to be true; we used biomimetic design principles to make PTDMs that are less toxic and more effective for moving cargo across the cell membrane than natural ones."

"What we modify is the chemical structure," Tew explains. "We've built the principles of a natural PTD into a new macromolecule that's bigger, longer, has more dense groups and different architecture. It slips into the T cell without damaging it, and can carry cargo, in this case siRNA, across that membrane."

They accomplished this by first identifying key features of natural PTDs, then capturing some of their chemical properties in new macromolecular synthetic polymer mimics. Specifically, they designed and studied two different PTDMs inspired by polyarginines and amphiphilic (able to cross both water and lipid membranes) peptides to deliver siRNA into two hard-to-transfect cell types: Cells from a line of immortalized human T lymphocytes and human peripheral blood cells from three different donors.

As one of several tests of effectiveness, they targeted knockdown of NOTCH1, a gene-controlling transmembrane receptor that also plays a role in T cell development, proliferation and differentiation. The authors report achieving 50 percent knockdown of NOTCH1 protein expression in both cell types for up to 72 hours after one treatment.

"The T cell goes about its business. It looks normal except that it has much less NOTCH1," says Tew. "With this new tool, we expect we can fine-tune T cell activation. In the case of cancer surveillance, you may want to turn it up to stimulate immune responses, but with autoimmune

diseases it would be beneficial to turn it down."

Provided by University of Massachusetts Amherst