Anticancer strategy is stuff of legend

Ovarian cancer succeeds in part by turning dendritic cells, important components of the immune system, into allies of the tumor. DMS scientists recently found a way to reprogram those cells, creating what the researchers describe as "Trojan horses."

Most cases of ovarian cancer are not diagnosed until the cancer has spread, and for patients with such a diagnosis the five-year survival rate is just 30%. One reason for that grim statistic is that the body's defenses often don't put up enough of a fight. "The established tumors that we treat represent failures of the immune system," says DMS immunologist José Conejo-Garcia, M.D., Ph.D.

Alert: Dendritic cells are key figures in that failure. When faced with a threat, such as a virus or a bacterial infection, dendritic cells alert the adaptive immune system, triggering a full-blown response from T cells. But ovarian tumors are able to transform dendritic cells, prompting them to promote angiogenesis—the creation of blood vessels that carry nutrients to the tumor. Even worse, the dendritic cells actively suppress an immune response, basically sending an all-clear signal when what's needed is a warning shot. "Rather than inducing antitumor immunity, they release signals that block it," Conejo-Garcia explains.

Mice treated with the targeted nanocomplex survived 40% longer.

When ovarian cancer spreads into the peritoneal cavity, many dendritic cells flock to that location, helping the tumor to grow. In an earlier study, Conejo-Garcia and other researchers in his lab showed that eliminating some dendritic cells from the peritoneal cavity led to a more effective immune response. So, he explains, "we thought, 'Well, what happens if rather than eliminating them we transform them.'"

Genes: To do that, the research team built a nanoscale combination of small interfering RNA (siRNA) and polyethylenimine (PEI, a molecule that is used to package and deliver the siRNA). The importance of siRNA—short, double-stranded lengths of RNA—has grown in recent years as scientists have figured how to use it to silence specific genes. Often it is aimed at tumor cells, turning off genes that help tumors grow. "But in our case, you target the dendritic cells," says Juan Cubillos-Ruiz, a graduate student in Conejo-Garcia's lab and the lead author of the paper that describes this recent finding.

Cubillos-Ruiz and Conejo-Garcia wanted to use siRNA to turn down the expression of the protein PD-L1. This protein causes the cells to issue chemical signals that suppress an immune response, clearing the way for the tumors to grow unchecked. They also knew that a side effect of using siRNA is that it can activate an immune response by stimulating certain receptors expressed on some immune cells, so they hoped that this byproduct might also prove beneficial.

The researchers say the experiments were surprisingly successful. When introduced into mice injected with an aggressive form of ovarian cancer, the siRNA turned down the expression of PD-L1. It also interacted with a receptor, TLR-7, that triggers dendritic cells to turn on other components of the immune system. In an unexpected development, PEI contributed to the reprogramming by interacting with a different receptor, TLR-5, likewise helping the cells fill their role in the immune response. Some of the dendritic cells even gained the ability to kill tumor cells directly, a phenomenon scientists first observed within just the past few years. So, Conejo-Garcia says, the nanocomplex turns dendritic cells into Trojan horses—cells that hide in plain sight and then attack the tumor cells.

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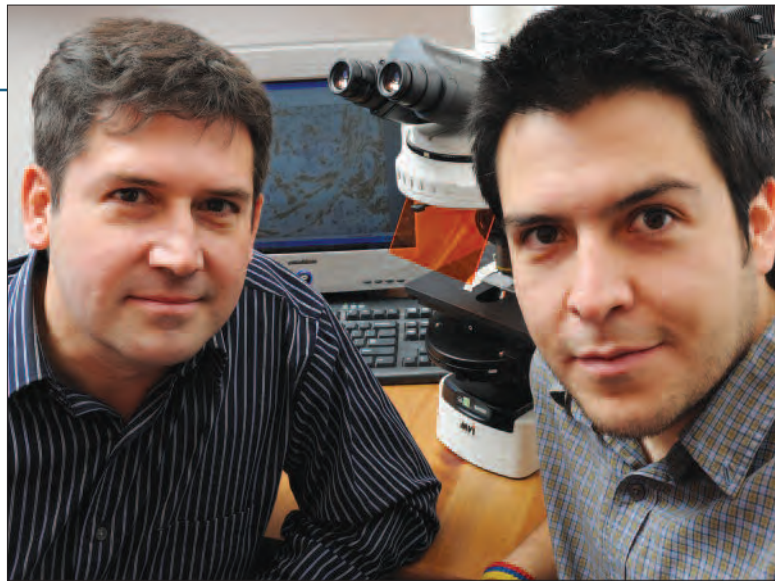

Conejo-Garcia, left, and Cubillos-Ruiz, right, call their strategy a "Trojan horse."

Conejo-Garcia and Cubillos-Ruiz found similar results when testing the effects of the nanocomplex on human cells taken from patients with advanced ovarian cancer. Next, they performed experiments to find out whether the nanocomplex could prolong life in mice with aggressive tumors.

One group of mice, the control group, was injected with saline. A second group received a version of the siRNA-PEI nanocomplex that did not target PD-L1. A third group received a version of the nanocomplex with siRNA targeted to turn off PD-L1.

Dramatic: The result, the researchers wrote in the *Journal of Clinical Investigation*, was "a dramatic increase in survival." Mice treated with the non-targeting nanocomplex had a 30% increase in survival time compared to mice in the control group. Mice treated with the targeted nanocomplex survived even longer, showing a 40% average increase compared to the control group.

So far, say Conejo-Garcia and Cubillos-Ruiz, there has been no sign of toxicity from the nanocomplex in mice, so now they hope to start the long process of turning their observations into improved treatments for humans. "We believe this could be tested right away," says Conejo-Garcia.

It won't be easy, of course, but after building Trojan horses, this team should be ready for the odyssey of clinical trials. AMOS ESTY