A novel cancer immunotherapy shows early promise in preclinical studies

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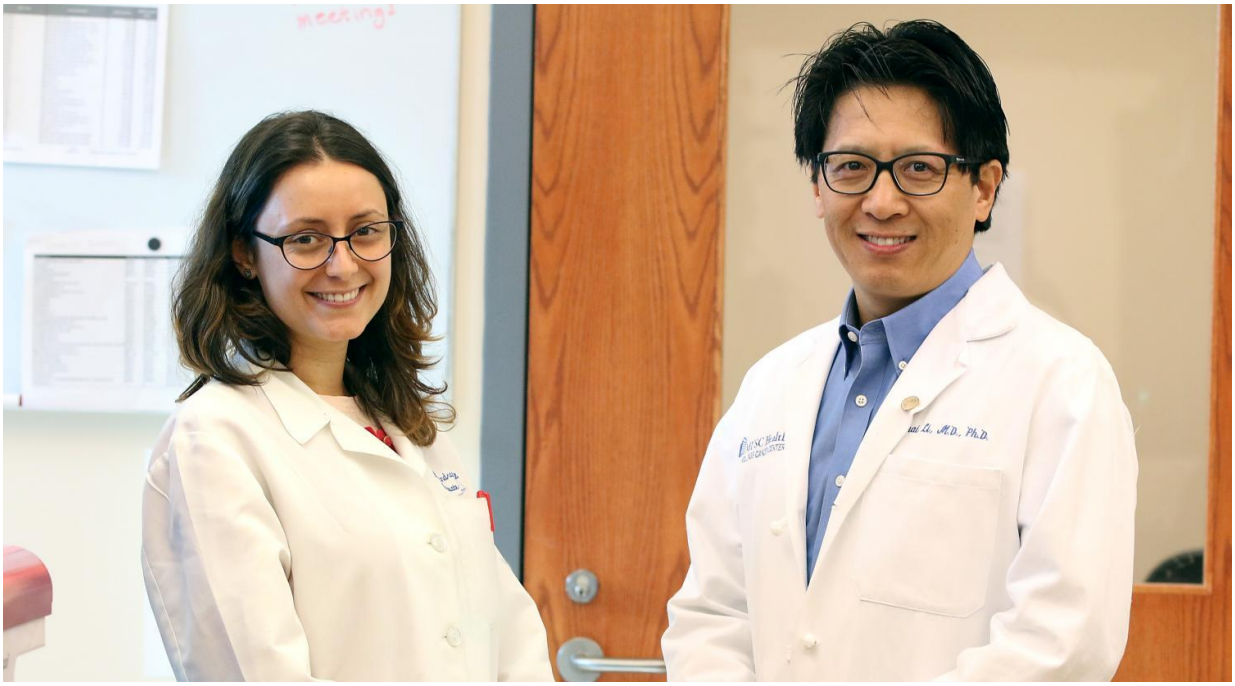

MUSC graduate student Alessandra Metelli (left) and Zihai Li (right), M.D., Ph.D., chair of the Department of Microbiology and Immunology at the MUSC Hollings Cancer Center, are first and senior authors, respectively, on a December 15, 2016 Cancer Research article showing early promise for GARP as a diagnostic and potential immunotherapeutic. Credit: Medical University of South Carolina

Scientists at the Medical University of South Carolina (MUSC) have

designed an antibody-based therapy that could target the functions of TGF-beta that cause cancer. The therapy targets TGF-beta where it is particularly dangerous—docked on the surface of tumor cells.

The team began by examining how TGF-beta grows out of control in the first place, according to Zihai Li, M.D., Ph.D., chair of the Department of Microbiology and Immunology at the MUSC Hollings Cancer Center and principal investigator on the project. "TGF-beta is an old story. The new spin is that there is a docking receptor for TGF-beta that increases the activity of the cytokine, and this molecule is called GARP," said Li.

TGF-beta is a cytokine, or secreted protein, that controls the cell cycle and is used by regulatory T cells (Tregs) as a signal to tell immune cells not to attack normal cells in the body. However, TGF-beta has become a widely studied cancer cytokine. Malignant tumors release large amounts of TGF-beta, which allows cancer cells to divide rapidly and to push Tregs to suppress immune cells that fight them. It has been uniquely difficult to design therapies that block TGF-beta, mainly because healthy cells cannot function without it.

Enter GARP. GARP is the only known receptor that allows TGF-beta to dock on the surface of cells. In this way, GARP helps cells to store TGF-beta. Importantly, Li knew that GARP could bind and activate TGF-beta and then float off the surface of cells that express it. Could this be a way that cancer cells store and release TGF-beta? The Li laboratory decided to find out.

In the December 15, 2016 issue of *Cancer Research*, Li and his colleagues, including first author and student Alessandra Metelli, report that levels of GARP were much higher in biopsies of human breast, lung, and colon tumors than in normal tissue. With this finding, it was reasonable to hypothesize that these higher levels of GARP provided more storage capacity for the TGF-beta needed for enhanced tumor

growth.

To examine if GARP had a direct role in cancer development, the MUSC team next deleted the gene for GARP from mice with mammary tumors and found that, without GARP, breast cancer tumors grew more slowly and were less able to metastasize to the lungs. Further experiments showed increased TGF-β signaling, tumor growth, and metastasis after the gene for GARP was inserted into mouse mammary tumor cells expressing high levels of GARP. Mice with more GARP also had more TGF-beta-releasing Treg cells. This meant that GARP promoted both cancer-intrinsic (metastasis) and -extrinsic (immune suppression) effects in breast cancer.

These were the first clues that GARP could be a diagnostic marker for cancer, according to Li. It also created an opportunity to develop new treatments.

The MUSC team immunized mice with human GARP in order to grow antibodies that could potentially block it. Only one antibody, 4D3, directly blocked human TGF-beta from binding to GARP expressed on cell surfaces. While 4D3 did not prevent growth of primary mammary tumors in mice, it did suppress the spread of these tumors to their lungs. However, 4D3 combined with cyclophosphamide chemotherapy curbed both primary tumor growth and metastasis. This means that combination immunotherapy with GARP antibody might boost the effectiveness of standard chemotherapy in breast cancer.

Li acknowledged that blocking GARP might also block the natural ability of Tregs to suppress the immune system, which could potentially lead to inflammatory autoimmune reactions. "Clinically some of the proven immunotherapies do induce some degree of autoimmunity," he said. "When cancer is cured and patients stop immunotherapy, the autoimmune manifestations completely disappear as well."

An accurate biomarker for GARP could offer an opportunity for earlier detection of other aggressive cancers, given that GARP levels increase before metastasis, according to Li. As part of their work, the group also showed that life expectancy was decreased in patients with colon or lung cancer whose biopsied tissues showed high levels of GARP. The challenge resides in the development of an antibody accurate enough to detect it in humans with cancer.

There have been great advances in cancer immunotherapies in the past decade, but there is still vast room for improvement. GARP suppression represents a novel addition to established cancer immunotherapies that also use antibodies to wake up the immune system to fight cancer.

"This discovery is fundamentally important to how TGF-beta utilizes GARP to promote cancer and down-regulate the immune system, but it also creates an opportunity for both diagnostics and therapeutics," said Li.

From the perspective of Li and his colleagues at the MUSC Hollings Cancer Center, they have only just scratched the cell surface.

More information: A. Metelli et al, Surface Expression of TGF Docking Receptor GARP Promotes Oncogenesis and Immune Tolerance in Breast Cancer, *Cancer Research* (2016). DOI: 10.1158/0008-5472.CAN-16-1456

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