Double trouble for triple-negative breast cancer: Two-pronged strategy restores immunotherapy sensitivity

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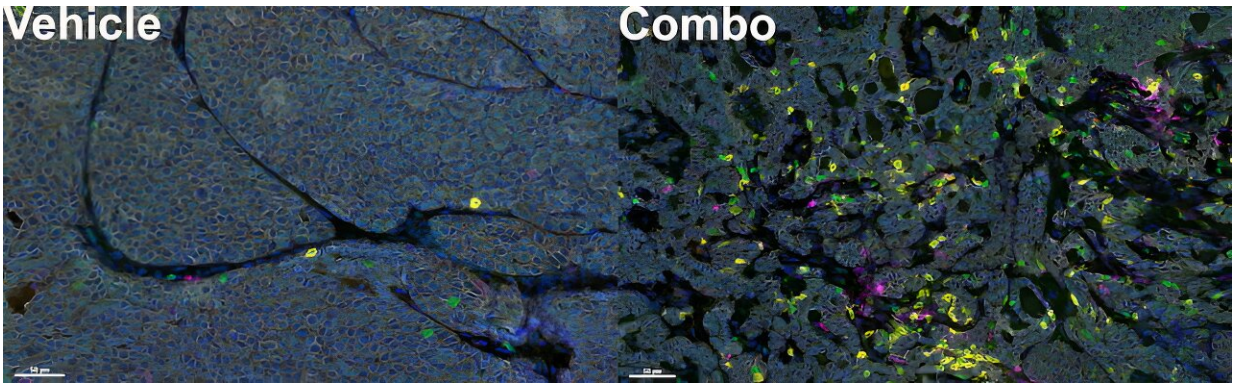

Tumor-fighting immune cells in the tumor before (left) and after (right) combination therapy. After combination therapy, many more tumor-fighting immune cells (T cells are shown in green and CD8+ T cells in yellow) have entered the tumor. Credit: *Cell Reports* (2024). DOI: 10.1016/j.celrep.2024.114532

A team of MUSC Hollings Cancer Center researchers has discovered one way in which triple-negative breast cancer (TNBC) cells become resistant to immunotherapy and have tested a two-pronged treatment strategy that was able to restore sensitivity to immunotherapy in a preclinical model.

The team, led by Besim Ogretmen, Ph.D., SmartState Endowed Chair in Lipidomics and Drug Discovery at MUSC, reports its findings in *Cell Reports*.

TNBC accounts for 10% to 20% of all breast cancer cases and is highly aggressive, with five-year survival rates markedly lower than for other breast cancers. It is twice as likely to occur in women under 40 than those older than 50 and is more common in Black women. TNBC is often diagnosed late, even after it has metastasized, making it difficult to treat.

TNBC earns its "triple-negative" moniker because patients with the disease already have three strikes against them. Because TNBC cells lack two necessary receptors, hormone-based therapies, a mainstay of breast cancer treatments, won't work. Neither will therapies that specifically target the protein HER2, as patients with TNBC lack it or have only very low levels. These three strikes leave patients with TNBC, particularly metastatic TNBC, with few treatment options.

"There's not a magic bullet target for triple-negative breast cancer," said Wyatt Wofford, an M.D., Ph.D. candidate in the Ogretmen Lab at MUSC and lead author of the article.

"Typically, response rates for TNBC are around 15% to 20%, and so that leaves about 80% of your patients who have metastatic TNBC with no good option for therapy."

In recent years, immunotherapy has proved to be a breakthrough therapy for many blood cancers but has had limited success in solid tumors like TNBC. The checkpoint inhibitor pembrolizumab has been approved for recurrent TNBC for patients with at least 10% of their tumor cells expressing the immunosuppressive protein PD-L1.

PD-L1 on the surface of cancer cells binds with PD-1 on the surface of immune cells known as T-cells, preventing them from doing their cancer-killing job and essentially slamming the brakes on the immune system. Checkpoint inhibitors release the brakes by preventing the binding of PD-1 and PD-L1, enabling the immune system to resume its attack on cancer.

The Ogretmen Lab studies sphingolipids, which are fat molecules that provide rigidity and stability to the cell membrane. One of the sphingolipids is ceramide. Enzymes synthesize a variety of ceramides, each with a different fatty acid chain length and a different role in cancer development and progression.

One of those enzymes, ceramide synthase 4 (CERS4), created ceramides that were important to maintaining membrane stability.

"The ceramide that this enzyme generates seems to be important to keep the tumor cell membrane intact so that everything stays where it needs to be," said Ogretmen. "When you lose this ceramide, the PD-L1 protein doesn't stay on the surface of the cells."

In their study, Ogretmen and Wofford found that when levels of that enzyme dropped and not enough of the ceramides it produced were available, the membrane became unstable, enabling PD-LI to drop down inside the tumor cell.

There, hidden inside the cell, PD-L1 was not exposed to the immunotherapeutic agent as it was while on the surface. With this finding, Ogretmen and Wofford identified a mechanism by which TNBC becomes resistant to immunotherapy. They also showed that the internalized PD-L1 could promote cellular pathways linked to metastasis.

The MUSC team next wanted to know whether they could make the tumor cells once again vulnerable to immunotherapy. The researchers treated a mouse model of TNBC lacking the enzyme CERS4 with both a PD-L1 inhibitor and an existing anti-cancer drug that blocks one of the metastatic cellular pathways promoted by the internalized PD-L1.

Before treatment, the mice uniformly developed lung metastases. However, after treatment, the cell membrane of the tumor regained its stability, and PD-L1 remained on the surface of the cell where it was exposed to immunotherapy and unable to promote tumor growth and metastasis.

Ogretmen and Wofford turned to Ozgur Sahin, Ph.D., SmartState Endowed Chair in the Department of Biochemistry and Molecular Biology, to do the drug studies. Wofford still remembers how excited he was when he first saw the results of these studies.

"When we looked at how the tumors were growing over time, use of immunotherapy by itself or the metastatic pathway inhibitor by itself did almost nothing. It was almost like you were giving the cancer cells water—they just weren't listening," he said. "And then, when you combined these two together, there was a really pronounced response. The tumors stopped growing and even started to regress."

For Ogretmen, the discovery of a biological mechanism for resistance in TNBC, although interesting in itself, is exciting largely because it will allow researchers to manipulate that mechanism to restore sensitivity to treatment.

"In this study, we were looking not only to study how TNBC becomes more resistant to immunotherapy but to use what we learned to make these cancer cells more responsive to immunotherapy," he said.

Currently, it is not feasible to move forward to the clinic with the pathway inhibitor tested in the study. The team's next steps are to identify other compounds, ideally existing, approved drugs that act on the same metastatic pathway and that could be used in a combination therapy with a PD-L1 inhibitor in patients with TNBC.

"We have some new leads and exciting results," said Ogretmen. "We are making progress at finding more viable combinations that we can actually take into the clinic."

More information: Wyatt Wofford et al, Alterations of ceramide synthesis induce PD-L1 internalization and signaling to regulate tumor metastasis and immunotherapy response, *Cell Reports* (2024). DOI: 10.1016/j.celrep.2024.114532

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