Researchers restore drug sensitivity in breast cancer tumors

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A team of Case Western Reserve University School of Medicine cancer researchers has uncovered one way certain tumors resist vital medication.

In the study published in *Oncotarget*, the researchers studied tumor biopsies collected from breast cancer patients before and after treatment with the go-to breast cancer drug trastuzumab (also known as Herceptin). Some of the tumors were treatable with trastuzumab, and others were not. By comparing genes activated in the responsive tumors to those in non-responsive tumors, the researchers uncovered several genes that may help tumors evade the drug. Tumors previously resistant to trastuzumab were resensitized when the researchers blocked one of the genes, called S100P.

The study zeroed in on small pieces of genetic material called mRNAs and lincRNAs. These tiny fragments are created from DNA inside normal cells but become dysregulated in tumors. The research team initially identified 1,542 mRNAs and 371 lincRNAs that were different between tumors cells responsive to trastuzumab and non-responsive tumors. These differences indicated to the researchers that separate networks of cell signals were being activated in each group of tumor cells. The researchers narrowed down the list of RNAs using cells grown in their laboratory. They were interested in finding an RNA molecule that could be therapeutically manipulated to disrupt signals in the tumor cells related to trastuzumab resistance.

Ahmad Khalil, PhD, Assistant Professor of Genetics at Case Western Reserve University School of Medicine led the study and explained, "Our hypothesis was that there are gene expression differences of both mRNAs and lincRNAs between tumors from patients that respond to trastuzumab and tumors from patients that do not."

Trastuzumab works by sticking to a protein called HER2 found on the surfaces of 25-30% of early-stage breast cancer tumor cells. The drug prevents HER2 from activating and controlling genes inside breast cancer cells. The research team grew breast cancer tumor cells with HER2 on their surfaces in their laboratory so they could validate findings from tumor biopsies. They exposed the cells to trastuzumab, mimicking cancer treatment regimens. Some breast cancer cells became resistant to trastuzumab after long-term exposure, just like the tumors collected from patients.

The researchers could identify mRNAs and lincRNAs that varied between trastuzumab-resistant and -sensitive HER2 cancer cells grown in the laboratory. They looked for overlap between the list of different RNAs in tumor biopsies and laboratory-grown cancer cells. The team identified 18 mRNAs and 7 lincRNAs that were associated with trastuzumab resistance in both models. The team zeroed in on a single gene that may be central to trastuzumab resistance after performing additional experiments in the laboratory.

The gene, S100P, is highly activated in breast cancer cells resistant to trastuzumab, as compared to normal breast tissue. Other studies have associated S100P with prostate and pancreatic cancers. It belongs to a family of genes that work together to support tumor growth and has been found in multiple compartments inside cancer cells.

"S100P was one of the key genes that showed significant expression differences," said Khalil. "It further stood out because it was part of a

pathway that emerged from a separate set of computational analyses of large datasets."

The researchers designed small pieces of genetic material to block S100P in breast cancer cells. Cells grown in the laboratory that were previously resistant to trastuzumab became sensitive to the drug after exposure to S100P blockers. Further analyses indicated that S100P activates critical proteins inside breast cancer cells to compensate for those turned off when trastuzumab blocks HER2. The activated proteins may help tumor cells adjust their gene expression in response to drugs in their environment.

"Our data demonstrated that high expression levels of S100P are required for cancer cells to become resistant to trastuzumab," concluded Khalil.

This exciting discovery indicates that depleting S100P in breast cancer may be one way to resensitize tumors to trastuzumab. The next step will be to further investigate the resistance mechanism, and screen for drugs that could be used to block S100P in human tumors. The researchers also plan to investigate the role of other mRNAs and lincRNAs from their list in regulating trastuzumab resistance.

Approximately one-third of early-stage breast cancer patients relapse after trastuzumab treatment, even if the drug is successful at first. Tumors in relapsed patients become resistant to trastuzumab which limits further treatment options. The mechanism behind trastuzumab resistance has not been easy to identify. Some studies have proposed mechanisms of trastuzumab resistance using cell culture models, but this study is the first to find mechanisms present in both cells growing in a laboratory dish and tumors growing in breast cancer patients.

According to Khalil, "Trastuzumab is a first line treatment for breast

cancer patients with HER2 gene amplification. Thus, finding the mechanism of resistance to this major drug now opens the door to reverse the resistance, potentially curing many more patients."

More information: Callie R. Merry et al, Transcriptome-wide identification of mRNAs and lincRNAs associated with trastuzumab-resistance in HER2-positive breast cancer, *Oncotarget* (2014). DOI: 10.18632/oncotarget.10637

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