Packaging therapeutic RNAs for targeted treatment of breast cancer

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(Medical Xpress) -- Researchers in the Program in Cellular and Molecular Medicine at Boston Children's Hospital and the Immune Disease Institute (PCMM/IDI) have developed a molecular delivery platform that overcomes one of the biggest obstacles to using RNA-based gene silencing technologies to treat cancer: Making sure the treatment gets to the right place and stays there.

Judy Lieberman, PhD, of the PCMM/IDI, and a former fellow from her laboratory, Erwei Song, MD, PhD, now at Sun Yat-Sen Memorial Hospital in Guangzhou, China, reported today in *Science Translational Medicine* that their platform—a combination of an antibody fragment and a packaging peptide—could successfully stabilize and deliver RNA-based molecules to tumors, silence them and prevent tumor growth and metastasis in a mouse model of breast cancer.

Breast cancer cells that display a protein called HER2 on their surface, which account for about 20 percent of all breast cancers, are highly aggressive. With the advent of targeted drugs like trastuzumab (Herceptin®) and lapatinib (Tykerb®), they have also become highly treatable. However, breast tumors that resist trastuzumab, relapse or do not have the HER2 protein can be quite difficult to treat.

To provide other targeted treatment options for breast cancers, Lieberman and Song have turned toa molecular phenomenon called RNA interference (RNAi), which relies on small pieces of RNA called small interfering RNAs (siRNAs) to silence the expression of individual

genes. Originally observed in plants, RNAi was found to be active in mammals only a decade ago, but it is already the focus of many clinical investigations.

A stumbling block in the clinical use of RNAi, however, is ensuring that the siRNAs get to where they need to go. "Naked siRNAs are cleared by the kidneys very quickly, and cells don't take them up readily," Lieberman explained. "Other delivery systems, like liposomes, tend to make siRNAs collect in the liver.

"The challenge is to deliver the siRNAs to the site of the tumor," she added, "without creating toxicity or inflammation in other portions of the body."

Lieberman and Song decided to adapt a targeting method they had originally developed for eliminating HIV-infected cells to transport siRNAs to breast tumors. First they bound siRNAs that would turn off a gene called PLK1 (an enzyme that promotes cell division) to protamine, a peptide that shrink-wraps DNA in sperm cells. Protamine was attached to an antibody fragment (called an ScFv) against HER2, creating a delivery package addressed directly to breast cancer cells with HER2 on their surface.

"The protamine stabilizes the siRNAs and protects them from being degraded by enzymes in the blood stream," Lieberman said. "And the ScFv antibody fragment makes sure that they only get delivered to and act in HER2-positive cells."

The team administered both naked and ScFv-protamine packaged siRNAs against PLK1 to mice with human breast tumors. While the naked siRNAs quickly accumulated in the kidneys and were excreted—their half life within the mice was a mere six minutes—Lieberman and Song found that the packaged ones were

delivered directly to the HER2-positive tumor sites within the mice. There they were readily detectable for at least 72 hours.

Once in the tumors, the siRNAs were absorbed into tumor cells, where they turned off PLK1, retarding tumor growth and suppressing metastasis compared to untreated controls or those that received naked siRNAs.

The team found no evidence of inflammation or toxicity in the liver, kidneys, or bloodstream, indicating that the siRNAs were silencing PLK1 only in their intended targets, the breast cancer cells.

"PLK1 is a ubiquitously expressed gene," Lieberman noted, "but because we targeted siRNA delivery only to HER2-expressing tumor cells, we could silence it with great specificity and no toxicity to other tissues."

Using the antibody-protamine delivery platform, the team could even deliver cocktails of siRNAs silencing different genes—AKT and CCND1, in addition to PLK1—for a synergistic effect against the mice's tumors.

Lieberman sees great promise for this technique for a broad range of cancers and other disease. "The platform could be used to target siRNAs therapeutically to any cell for which we have a cell surface marker to target. We have also used it to target lymphocytes, suggesting it could be useful against lymphomas and leukemias and as a way of countering organ rejection."

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