Non-coding portions of genome are found to play role in cancer

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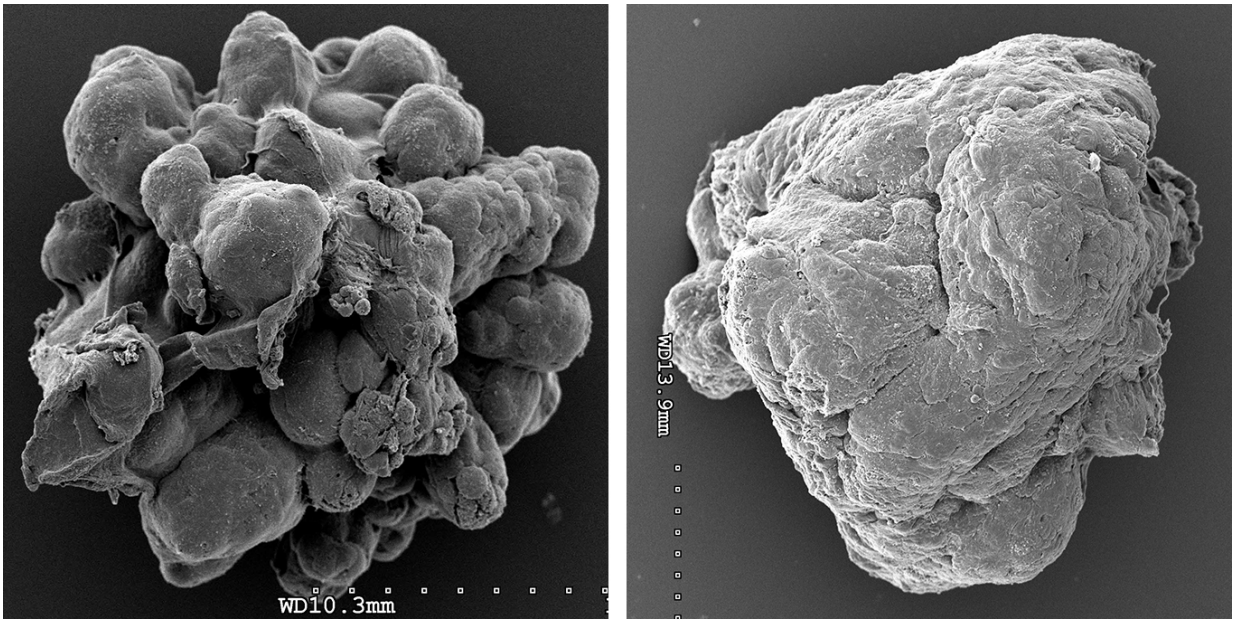


Members of the Spector Lab at CSHL grew breast cancer organoids—clumps of cells sampled from specific cancer subtypes, in this case luminal B—and observed them as they expanded, exhibiting properties typical of aggressive, metastatic human cancers. The organoid on the left reflects aggressive luminal B breast cancer in its clumpy, bulbous appearance, somewhat like an expanding bunch of grapes. Treatment with an antisense molecule called ASO#4 knocked down a long non-coding RNA called MaTAR20 that is over-expressed in such cancers. After 6 days of treatment, the organoid is smaller in size (not apparent here) and has lost its clumpy appearance, indicating a loss of growth characteristics associated with metastasis. Electron microscope images by Stephen Hearn, CSHL. Credit: Cold Spring Harbor Laboratory

The human body produces 100,000 or more different proteins. Yet, amazingly, only two percent of the human genome actually encodes proteins. Nearly 80 percent of the rest of the genome is transcribed into RNA that does not code for proteins. Two big questions facing scientists are: How much of this "non-coding" RNA is actually functional? And does it play a role in disease?

A team of scientists at Cold Spring Harbor Laboratory (CSHL) screened thousands of non-coding RNAs to find those that were expressed at high levels in two types of aggressive breast cancer. As they describe today in a paper appearing in *Cell Reports*, when they reduced the level of some of the most over-expressed of these RNAs from mammary tumor samples, cellular features characteristic of cancer spread were significantly reduced.

Of the handful of different types of non-coding RNA, the most abundant and least understood are long non-coding RNAs, or lncRNAs. About 16,000 lncRNAs have been identified in humans, but functions for the vast majority are unknown.

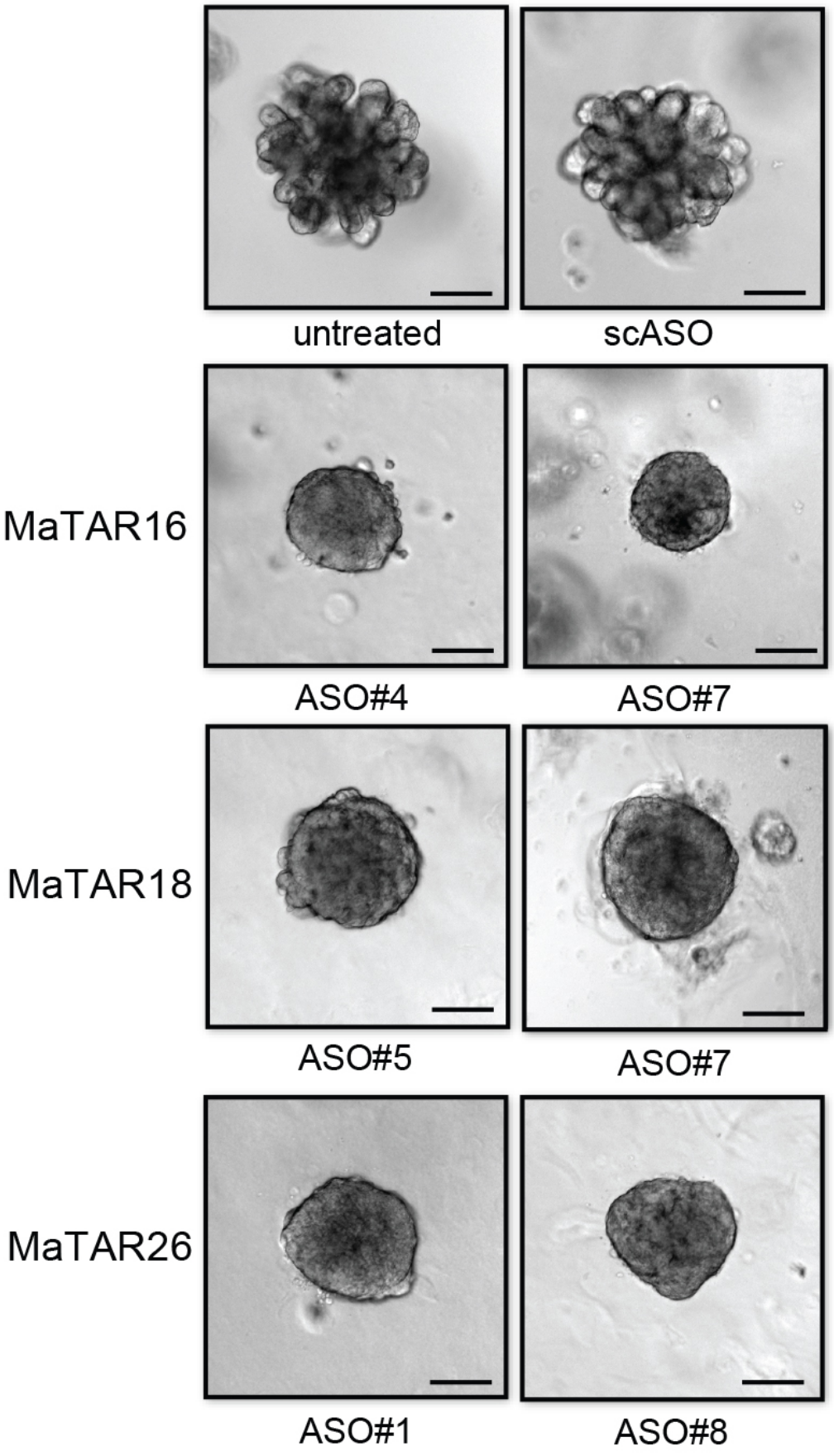
"Since so much of the genome is being transcribed into RNA, it would seem that there would be a vast wealth of potential therapeutic targets out there that have not really been studied," says the team leader, CSHL Professor David Spector, who is also Director of Research at the Laboratory.

While the exact functions of most lncRNAs remain to be discovered, it has already been shown that in some cases their over-expression is linked to specific cancers, including breast cancer, prostate cancer and leukemia. Earlier this year, Spector's team demonstrated that a lncRNA called *Malat1* was a critical regulator of breast cancer progression. Eliminating that particular lncRNA in a mouse model of luminal B breast cancer caused the cells within the primary tumor to change

character, and resulted in a significant reduction in metastasis.

"That study provided significant motivation for us to look for other lncRNAs that might also be over-expressed and impact breast cancer," says Spector.

Spector and his team, led by postdoctoral fellow Sarah Diermeier, systematically sifted through the vast database of lncRNAs to identify those that are expressed more often in tumors, relative to normal mammary cells.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Top line: a luminal B breast cancer organoid prior to treatment (left), and after treatment with an antisense molecule, or ASO, intentionally designed to "miss" key long non-coding RNA targets. Compare the next 3 rows, in which different ASOs designed to target 3 different long non-coding RNAs overexpressed in this breast cancer type, act to different degrees to limit metastatic characteristics of breast cancer organoids. Overall, ASO#7, targeting MaTAR16, is most effective in shrinking a "metastatic" organoid while also changing its surface appearance from bulbous to smooth. Credit: Stephen Hearn, CSHL.

The team found several hundred lncRNAs that were expressed at higher than normal levels in both types of aggressive mouse tumors that they tested: luminal B and Her-2 positive. They then performed an extensive computational analysis to prioritize a subset of 30 of these lncRNAs that they dubbed Mammary Tumor Associated RNAs, or MaTARs.

In collaboration with Ionis Pharmaceuticals, Spector and hcolleagues designed a series of molecules that bind tightly to, and thereby destroy, specific RNA sequences. They used these so-called "antisense" molecules to wipe out individual MaTARs in mammary cancer-derived organoids, three-dimensional models of tumor cells that represent many features of real tumors.

The researchers found that individually eliminating 20 of the 30 MaTARs in these organoids diminished features associated with cancer, including cell proliferation, invasion, and migration.

"We now have an innovative way of destroying RNA targets inside live cells and assessing whether a tumor is dependent on them for survival," says Spector.

The team's next step is administering antisense molecules to degrade specific MaTARs in mice, in the hope that this will decrease primary tumor mass and/or metastasis. Should those experiments be successful, Spector's team will perform additional preclinical tests in human tumor samples to better identify which subgroups of patients would benefit most from being treated with antisense molecules to eradicate certain lncRNAs or clusters of lncRNAs.

"We think these tests will have particular relevance for personalized medicine," says study first author Diermeier "We imagine a situation where organoids can be derived from an individual's tumor, grown up in a dish, and act as a platform for figuring out which antisense molecules comprise the optimal treatment for a patient."

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"Mammary Tumor-Associated RNAs Impact Tumor Cell Proliferation, Invasion and Migration," appears online September 27, 2016 in *Cell Reports*. The authors are Sarah D. Diermeier, Kung-Chi Chang, Susan M. Freier, Junyan Song, Osama El Demerdash, Alexander Krasnitz, Frank Rigo, C. Frank Bennet, and David L. Spector. The paper can be viewed at http://www.cell.com/cell-reports/newarticles

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