Finding A Way to “Switch” Off the Spread of Breast Cancer

The National Cancer Institute recommends that women age 40 and older have a mammogram every one to two years as routine early detection for breast cancer. This is because the best way to prevent the growth and spread of cancer is to find the disease as early as possible and remove or destroy the cancerous cells.

Once breast cancer has spread, treatments can delay progression of the disease and prolong survival times but it is not a curable condition. Complications involving the spread, or metastasis, of a primary tumor remain the greatest cause of mortality from breast cancer.

For this reason, researchers have been trying to determine exactly how breast cancer cells invade surrounding tissues and spread. If these processes are understood, the researchers can develop interventions to prevent or limit breast cancer from spreading.

Until a few years ago, Dr. Anthony J. Koleske, Yale School of Medicine Professor of Molecular Biophysics and Biochemistry, was not involved in breast cancer research. He and his laboratory colleagues had been studying how cells change shape and move to spread into surrounding tissue – in the healing of wounds. He had identified particular proteins – essential building blocks of cells – which play critical roles in what he calls this “shape-shifting” process of cell movement.

As he reviewed the literature on these proteins, Koleske became aware that one of the proteins he had identified appeared to be a lynchpin in the formation of protrusions from cancer cells. These protrusions enable cancer cells to degrade and penetrate their outer membranes from within, to begin the invasion of surrounding tissue. Called invadopodia, these protrusions play a key part in the metastasis of breast cancer, which after lung cancer causes the greatest cancer mortality in women.

“We decided to move from studying cell movement in wound healing to these invadopodia,” Koleske said in a recent interview, “because they engage even more pressing health concerns.”

His further research showed that three particular proteins come together to form a control “switch” in breast cancer cells which enables them to poke their way through a membrane and invade surrounding tissues, where they can develop secondary tumors, in blood vessels and other tissues such as the lungs – a common site for the spread of breast cancer.

Dr. Koleske teamed with Dr. Titus J. Boggon, Associate Professor of Pharmacology, and as Co-Principal Investigators the two scientists were awarded a 2011 Pilot Project Program grant from Women’s Health Research at Yale to study this switch and how to keep it from forming. By determining how to disrupt the assembly of these three proteins, they will take important first steps toward developing a new class of drugs that could target this switch and thus limit the spread of breast cancer cells. Currently, there are no drugs that selectively target breast cancer invasion or metastasis.

“We’re delighted that this work has come together...
and we’re excited by this study,” Koleske said. “We have a long way to go, involving a lot of work and time, yet we are cautiously optimistic that this might turn out to be a critical approach to reducing or preventing metastasis.”

Interlocking Legos
To envision the control switch, Koleske suggests picturing the three proteins as interlocking Lego building blocks. In previous laboratory work, Koleske determined that knocking out one or more of these blocks in aggressive human breast cancer cells hampers the cells’ invasive capacity and disrupts their ability to metastasize to the lung in an animal model. “We’ve done enough research to establish proof of principle to know we can make an invasive cell into a non-invasive cell by inhibiting the switch,” Koleske said.

In this new pilot study, co-funded by Women’s Health Research at Yale and Yale Cancer Center, the goal is to identify small-molecule compounds that can disrupt the assembly of the breast cancer invasion switch in living organisms. Many approved drugs are small molecule compounds, characterized by low molecular weight and small size, and a high affinity for binding to proteins and altering their function – traits that are expected to be crucial in disrupting the switch.

Searching for compounds that will work
To find candidates for drug development, the team will use the Yale Small Molecule Discovery Core Facility, on Yale’s West Campus straddling West Haven and Orange, to screen thousands of these compounds to identify those with the most potential. Under the direction of Dr. Janie Merkel, Director of Biology, this facility has an inventory of more than 140,000 compounds, both natural and man-made, with varying traits, and employs the latest instruments for rapid screening of hundreds of these compounds at once.

Although there is no guarantee that this study will identify a drug, chances are good that some compounds that work will be found, and can be improved, Koleske said. This is where Dr. Boggon’s laboratory comes into play. “Our lab will test how the compounds interact with the proteins,” Koleske said. “His lab will figure out how to make the compounds work better.”

Terminology & Definitions

Breast Cancer Metastasis: Spread of cancerous cells from breast tissue to nearby lymph nodes or other parts of the body. Complications from the spread of a primary tumor are the greatest cause of mortality from breast cancer.

Proteins: Building blocks, or fundamental components, of all living cells. Three proteins are believed to come together to form a control “switch” – identified by Dr. Anthony Koleske – that turns on the invasiveness of breast cancer cells.

Invadopodia: Protrusions in cell membranes that, in breast cancer cells, are enabled by the control “switch” to begin the invasion of surrounding tissue. The invasion and spread of these cells leads to the formation of secondary tumors, and if unchecked, can lead to metastasis.

X-Ray Crystallography: Method of visualizing the arrangement and structure of atoms within a crystal – in which x-rays pass through the crystal and bend, or diffract, in different directions. A three-dimensional picture can be produced from the diffraction pattern.

Structure-guided Drug Design: Method for designing drugs based on detailed knowledge of the three-dimensional structure and the functions of cell components or proteins that are the intended targets.
This work takes place literally under a microscope - at the molecular level of the proteins and candidate compounds. One of Boggon’s areas of expertise is turning the proteins into crystal form, so that their structures can be visualized using a technique known as x-ray crystallography. In this process, x-ray beams pass through and bounce off atoms in the crystals, causing the light to bend, or diffract, in different directions. The diffraction pattern is used to discern the three-dimensional details of the molecules under study.

“This will allow us to see the precise structure of the proteins and how the surfaces lock together,” Boggon said. “When we can see – in very high resolution – how the machinery works, we can improve the thing that will jam the machinery.”

One of Boggon’s main aims will be to identify the exact sites where the inhibiting compounds stick to the proteins involved in the breast cancer invasion switch, knowledge which will be critical in improving their disruptive capacity. Once this capacity is optimized, Koleske and Boggon will then test the efficacy of the compounds in disrupting breast cancer invasion using cell cultures in the laboratory.

The two scientists ultimately will use the results of their pilot study to apply for a larger grant from the National Institutes of Health to continue their work on developing a therapy to block the spread of breast cancer cells.

Their longer-term goal is to design a drug based on what they discover from understanding the structures and assembly of the proteins that form the breast cancer invasion switch, and the structures and functions of the inhibiting compounds that they identify and optimize.

They envision in the not too distant future conducting clinical trials with a drug developed through this structure-guided design. The drug would be for use as follow-up therapy after a primary breast cancer tumor is treated with surgery, chemotherapy and radiation.

### About the Investigators

**Anthony J. Koleske, Ph.D.** is Professor of Molecular Biophysics and Biochemistry and Professor of Neurobiology. He received his B.S. from the University of Wisconsin and his Ph.D. from the Massachusetts Institute of Technology. His areas of research interest include understanding how nerve cells develop and deteriorate, cell migration, cancer and metastasis.

**Titus J. Boggon, Ph.D.** is Associate Professor of Pharmacology. He received both his B.S. and his Ph.D. from the University of Manchester, U.K. His research interests include understanding mechanisms that cells use to send and receive signals using interconnected molecules, and applying such information to drug discovery.