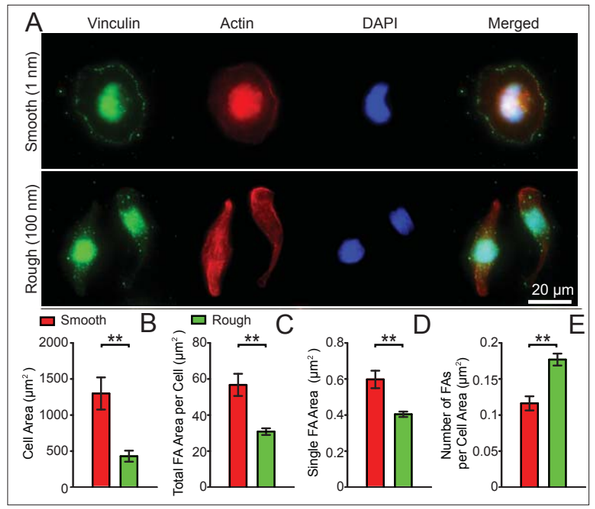

Improving Cancer Detection, Prognosis and Monitoring

Cancer cells that "break away" from tumors and travel through the blood to other parts of the body can be deadly. In fact, 90 percent of cancer-related deaths stem from metastasis. These circulating tumor cells (CTCs) have been the subjects of much clinical and biomedical research in recent years, since CTCs can serve as excellent diagnostic and prognostic markers, sometimes replacing invasive biopsy procedures.

"The CTCs have been shown to be directly connected to patient outcomes," said **Jianping Fu**, assistant professor and director of the Integrated Biosystems and Biomechanics Laboratory. "They clearly offer a window of opportunity for patients and also to those of us interested in studying the biological nature of cancer metastasis." How do CTCs circulating in the blood differ from the cancer cells in solid tumors? Why do these cells break away and travel? What strategies can be used to block the escape process of CTCs? "Those answers would provide insights for future therapeutic interventions and personalized treatment approaches," Fu said.

Capturing and isolating CTCs from a blood sample is not without major challenges, however. The CTCs are typically present in the blood in low abundance, on the order of one CTC per billion blood cells.

"Circulating tumor cells are a problem of great clinical and biomedical significance, but the technical issues related to how to capture CTCs from unprocessed or minimally processed blood samples are very challenging," Fu said.

The most common method for isolating CTCs today relies on the use of capture antibodies that recognize specific antigens expressed on the surface of CTCs. But scientists are finding that CTCs are much more heterogeneous than previously thought, and "using capture antibodies will miss some sub-populations of CTCs that don't express those target surface antigens. Researchers believe those subgroups of CTCs might be cancer stem cells or cancer progenitor cells that lead to secondary tumors and therefore are the most dangerous," Fu said. "We need a strategy that can capture CTCs, regardless of their surface marker expression."

Fu and his research team have developed such a strategy. His approach is based not on CTC phenotype—such as surface marker expression or cell size, which scientists also have used to try to capture them—but on function: since they form tumors, CTCs are necessarily adherent. In stark contrast to blood cells, they attach readily to surfaces, and their adhesion strength is high.

To leverage CTCs' adherent properties, Fu is using a common nanofabrication technique, reactive ion etching, to precisely pattern and roughen a glass surface. Blood samples placed on the surface are incubated and analyzed. The CTCs in the blood sample begin to attach to the roughened surface almost immediately, and Fu has found that more than 90 percent of CTCs in the blood sample can be captured.

Others have observed that nanorough surfaces can enhance CTC capture, but Fu's research group is the first in the world to do so *without* using capture antibodies, as explained in a cover page article published in the American Chemical Society journal *ACS Nano* (DOI: 10.1021/nn304719q).

The simplicity of Fu's approach paves the way for larger-scale fabrication of nanorough surfaces, a critical component for mainstream clinical use and chip-based and/or disposable diagnostic and prognostic devices. "Our strategy would provide a unique advantage," he said.

The team is conducting further experiments on different cell lines and types of cancers. Fu currently is working with collaborators from the U-M Medical School and testing preclinical and clinical blood samples. Preliminary results using preclinical blood samples suggest his approach is effective. "Our strategy can capture CTCs with almost uniform performance regardless of the surface protein expression of cancer cells," he said.

Going forward, Fu plans to use some of the micro- and nanofluidic tools his research group has developed to isolate and analyze low-abundant CTCs cells from preclinical and clinical blood samples in order to study the biophysical properties as well as surface protein expression patterns of CTCs. "These tools enable biochemical and biomedical phenotyping of CTCs," he said. "We have the capability to examine the biological nature of CTCs down to a single-cell resolution."

The work has been supported by the U-M Comprehensive Cancer Center, the Michigan Institute for Clinical and Health Research and the National Science Foundation.

"It may be a simple strategy," Fu said, "but it provides the opportunity to learn much more about the metastatic process. For the first time, clinicians and cancer researchers will have access to cancer cells as they transition from primary to secondary tumors. It's very exciting."

> "Our strategy can capture CTCs with almost uniform performance regardless of the surface protein expression of cancer cells."

OPPOSITE PAGE: **Effect of nanotopological sensing on focal adhesion (FA) formation of MDA-MB-231 cells. (A) Representative immunofluorescence images of MDA-MB-231 cells adherent on smooth (Rq = 1 nm) and nanorough (Rq = 100 nm) glass surfaces after 24 h of culture. Cells were co-stained for nuclei (DAPI; blue), actin (red), and vinculin (green). (BE) Cell area (B), total FA area per cell (C), average single FA area (D), and number of FAs per cell area (FA density; E) of MDA-MB-231 cells adherent on smooth (Rq = 1 nm) and nanorough (Rq = 100 nm) glass surfaces after 24 h of culture. Error bars represent (s.e.m. (n > 30). Statistical analysis was performed by employing the Student's t-test. Double asterisk (//) indicates p < 0.01.**

RIGHT: **Capture and enumeration of cancer cells. (A) Representative fluorescence and merged microscopic images showing known quantities of MDA-MB-231 cells as indicated spiked in lysed blood captured on nanorough glass surfaces (Rq = 150 nm) 1 h after cell seeding. Target MDA-MB-231 cells were labeled with CellTracker Green before spiked in lysed blood that was prestained with DiI. (BE) Regression analysis of 1-h capture efficiency for MCF-7 (B,C) and MDA-MB-231 (D,E) cells on smooth (Rq = 1 nm) and nanorough (Rq = 150 nm) glass surfaces. In panels AE, known quantities of cancer cells (n = 101000) were spiked in 500 μL of growth media containing PBMCs or 500 μL of lysed blood as indicated. For PBMC samples, cancer cells were mixed with PBMCs at a constant ratio of 1:1. Insets in B and D show correlations between captured cell number and loaded cell number for n = 1060, indicating efficient capture of low abundant CTCs. Solid lines represent linear fitting. Error bars represent (s.e.m. (n = 4). of MDA-MB-231 cells to PBMCs. Results were obtained 1 h after cell seeding. In panels AD, a fixed number of cancer cells (1000) were mixed with PBMCs in growth media to achieve cell ratios from 1:1 to 1:200. Error bars represent (s.e.m. (n = 4).**