Microfluidics-imaging platform detects cancer growth signaling in minute biopsy samples

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Inappropriate growth and survival signaling, which leads to the aberrant growth of cancer cells, is a driving force behind tumors. Much of current cancer research focuses on the kinase enzymes whose mutations are responsible for such disregulated signaling, and many successful molecularly targeted anti-cancer therapeutics are directed at inhibiting kinase activity.

Now, UCLA researchers from the Crump Institute for Molecular Imaging, the Institute for Molecular Medicine, the California NanoSystems Institute, the Jonsson Comprehensive Cancer Center and the department of molecular and medical pharmacology have developed an in vitro method for assessing kinase activity in minute tissue samples from patients. The method involves an integrated microfluidics and imaging platform that can reproducibly measure kinase enzymatic activity from as few as 3,000 cells.

In a paper published Nov. 1 in the journal *Cancer Research,* the UCLA researchers describe several new technological advances in microfluidics and imaging detection they co-developed to measure kinase activity in small-input samples. The team applied their microfluidic kinase assay to human leukemia patient samples.

"Because the device requires only a very small tissue sample to give results, this method creates new potential for direct kinase

experimentation and diagnostics on patient blood, bone marrow and needle biopsy samples," said lead investigator Thomas Graeber, a UCLA professor of molecular and medical pharmacology. "For example, the stem cell properties of leukemia can be directly studied from patient samples."

To improve radio-signal detection, the team used a novel imaging detector, in the form of a solid-state beta camera, which can sensitively detect and spatially resolve radioactive signal directly from a microfluidic chip. The beta camera provides a picture of the activity on the chip, allowing real-time monitoring of the assay performance and outcome. It is highly sensitive and quantitative.

In their first application of the device, the team measured the activity of the mutated kinase responsible for chronic myelogenous leukemia. This mutation is targeted by the clinically successful kinase inhibitor Gleevec.

"We are not aware of other work demonstrating solid-state integrated radioactive imaging from a microfluidic platform," said co-investigator Arion Chatziioannou, a UCLA professor of molecular and medical pharmacology.

The resulting microfluidic in vitro kinase radioassay improves reaction efficiency, compared with standard assays, and can be processed in much less time. This greater efficiency, coupled with the high sensitivity of the beta camera, reduces the amount of sample cell input by two to three orders of magnitude, compared with conventional and 96-well assays. The assay includes a kinase immunocapture step to increase specificity towards the kinase of interest.

"To get the kinase assay to work in a microfluidic environment, we needed to develop new protocols and reagents for efficiently manipulating solid-support kinase capture beads using microfluidic trap-

and-release valves," said co-investigator Hsian-Rong Tseng , a UCLA professor of molecular and medical pharmacology.

"Integration of the solid-state beta camera allows researchers to monitor the assay in real time, which proved useful during our protocol development and testing," said Cong Fang, the leading graduate student on the project. "The integrated microfluidic and imaging platform opens new possibilities and makes miniaturization of many common radioactivity-based bioassays to the microfluidic realm possible."

"With the integration of the compact camera, the microfluidic format assay has the potential to be developed into inexpensive bench-top, stand-alone units," said UCLA postdoctoral fellow Nam Vu, who led the imaging development.

"Taken together, the reduced sample input required, the decreased assay time, and the digitally controlled reproducibility of the team's microfluidic kinase radioassay facilitates direct experimentation on clinical samples that are either precious or perishable," said UCLA postdoctoral fellow Yanju Wang, who led the design of the network of microfluidic components that run the assay.

Future experiments will develop reproducible sample collection and measurement conditions for primary patient samples.

Other applications could include profiling of patient and animal model samples for their kinase-inhibitor drug sensitivity, or measurement of kinase activity from stem cells, cancer stem cells and other rare immune cells.

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