Genome sequencing method can detect clinically relevant mutations using five CTCs

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Whole genome sequencing using long fragment read (LFR), a technology that can analyze the entire genomic content of small numbers of cells, detected potentially targetable mutations using only five circulating tumor cells (CTCs) in a patient with metastatic breast cancer.

The study is published in *Cancer Research*, a journal of the American Association for Cancer Research, by Brock Peters, PhD, senior director of research at Complete Genomics Inc. in San Jose, California, and BGI-Shenzhen in Shenzhen, China; John W. Park, MD, professor of clinical medicine, and director of Novel Therapeutics, Breast Oncology, at University of California, San Francisco (UCSF); Hope S. Rugo, MD, professor of medicine and director of breast oncology and clinical trials education at UCSF.

The Complete Genomics team and colleagues from UCSF evaluated CTCs from two liquid biopsies drawn from a 61-year-old female patient with ER-positive/HER2-negative metastatic breast cancer at two different time points during her course of treatment. First, they isolated 34 highly pure CTCs using immunomagnetic enrichment/fluorescence-activated cell sorting (IE/FACS) technology developed by Park and Mark Magbanua, PhD, at UCSF. Then they used LFR to perform advanced whole-genome sequencing by splitting the genomic DNA from the CTCs into 3,072 individual compartments, with each compartment containing approximately 5 percent of the cancer genome. The DNA in each compartment was subsequently labeled with a unique barcode, the compartments were combined, and the genomic DNA and barcodes

were sequenced.

"From 34 cells we accurately detected mutations present in as few as 12 percent of CTCs, established the tissue of origin, and identified potential personalized combination therapies for this patient's highly heterogeneous disease," said Peters.

According to Peters, this research is the first application of LFR technology to CTCs. "LFR subdivides the genome into compartments, allowing us to count the fragments with somatic mutations across all the compartments to accurately quantify the number of mutations present in a population of cells. It also serves to remove false-positive single nucleotide variants," explained Peters.

"LFR, which explores the more than 20,000 genes in the genome and all non-coding regions, is more comprehensive than gene panels, which examine about 100 genes and focus on small genomic regions typically associated with a disease," he continued.

Because prior studies indicate that five CTCs can be expected in about half of the patients with metastatic disease, and evaluating 34 CTCs is cost-prohibitive, Peters and colleagues analyzed five different batches of five CTCs and replicated their findings. The researchers estimated that the cost of their advanced whole genome sequencing technique on five CTCs would be about $3,000 within the next few years, in line with current oncology diagnostic tests.

"That our sequencing method could detect the most important somatic mutations from just five CTCs in a noninvasive liquid biopsy is important, demonstrating cost-effectiveness and utility in clinical settings," said Peters.

"Our work highlights the importance and utility of using accurate and

quantitative whole genome analysis in a clinical setting," said Peters. "We identified targetable mutations that would have been missed by current clinical sequencing strategies. In the near precision medicine future, this type of information will be critical for selecting effective personalized multi-drug treatments."

Study co-author John W. Park, MD, professor of clinical medicine, and director of Novel Therapeutics, Breast Oncology, at University of California, San Francisco (UCSF), said, "We observed that it is possible to develop a robust strategy for liquid biopsy using whole genome sequencing of circulating tumor cells. This approach allows detailed molecular profiling across the patient's entire cancer genome."

Study co-author, Hope S. Rugo, MD, professor of medicine and director of breast oncology and clinical trials education at UCSF, said, "The IE/FACS allows for exquisite and full-scale isolation of highly pure CTCs with little or no contamination of normal blood cells, thus providing the robustness needed for accurate whole genome sequencing of a few cells. Taken together, the liquid biopsy platform we described in this study suggests a viable approach for minimally invasive yet comprehensive and real-time testing of metastatic cancer in the clinic."

According to Peters, the main limitations to the study are that only a single patient was studied and none of the suggested possible therapies could actually be tested, emphasizing the need for larger studies.

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