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New technology isolates pure tumor cells from FFPE samples

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Using the innovative DEPArray technology, scientists at Silicon Biosystems, a Bologna (Italy) and San Diego (CA, USA) based biotech company, were able to solve one of the biggest limitation in the study of cancer genetic: tumor samples heterogeneity.

The study, published February 11, 2016 by *Scientific Reports*, presents, for the first time, a revolutionary method to isolate 100% pure tumor and stromal cell populations from minute formalin fixed, paraffin embedded (FFPE) specimens allowing downstream analysis of tumor genetic characteristics via next generation sequencing (NGS) with unprecedented precision.

The application of precision medicine in oncology requires a rigorous understanding of the genetic characteristics that drive tumorigenesis. Available technologies, like NGS, hold an incredible potential but their accuracy is limited by the fact that biopsied tissue specimens represent a mixture of different cell types present in different proportion.

Moreover heterogeneity inside the tumor cell population itself limits the possibility to identify tumor drivers, as low represented clones may actually be the one responsible for more malignant traits. Sample heterogeneity can thus affect the performance of powerful downstream molecular analysis by hiding genetic variants due to the dilution of samples.

Current available technology to solve samples heterogeneity, like laser

capture microdissection and FACS sorting, still lack the accuracy and purity required for clinical implementation and their power is often limited by the size and quality of the starting sample materials.

Starting from a wide collections of FFPE clinical samples of different size and with different tumor cellularity, researchers at Silicon Biosystems used the DEPArray cell sorting system to digitally separate precise numbers of pure, homogeneous cells based on their marker phenotype and DNA content. Distinct tumor populations with different DNA content (diploid and hyperdiploid fractions) could be separated in purity from the diploid stromal fraction as demonstrated by results of downstream targeted NGS.

When comparing variant frequencies between pure-DEPArray sorted populations researchers could assign, unambiguously and quantitatively, the genetic variants to the different classes of cells (stromal population, diploid or hyperdiploid tumor populations). On the contrary targeted NGS results from the unsorted fraction gave a blurred picture of the genetic profile of the tumor and didn't allow detection of somatic mutations or loss of heterozigosity (LOH).

The researchers also confirmed the results by low-pass whole genome sequencing (WGS). The copy-number profiles of sorted pure populations confirmed the copy number variations (CNVs) and LOH events predicted with targeted NGS and further allowed to interpret dual events on the same locus, like mutation and LOH. The power of the DEPArray cell sorting was further demonstrated using FFPE specimens with low tumor cellularity (5%), which are usually inaccessible to accurate genetic analysis and are associated with poor patients' prognosis. The low-pass WGS profile of sorted pure tumor cells from these samples allowed identification of numerous gain and losses along the genome in contrast to the analysis of the unsorted sample, where the signal from tumor cells was so diluted by the contaminating stromal

DNA that most of the gains and losses were undetectable.

"The method we have developed, based on a pre-analytic digital cell separation from FFPE samples, achieves 100% purity of the sample, substantially reverting the DNA composition to a germline-like situation where NGS techniques can display all their power and reliability," states Nicolò Manaresi, lead author of the paper. "Data analysis and interpretation of results is also drastically simplified and different classes of genetic alterations can be resolved with unprecedented precision".

The paper shows that the DEPArray cell sorting technology, followed by NGS analysis, reveal comprehensive genomic information from any FFPE sample, regardless of tumor cellularity and size of the specimen, and has the potential to revolutionize translational cancer research, including biomarker discovery, and setting a new gold standard for accuracy in oncology precision medicine.

More information: Chiara Bolognesi et al. Digital Sorting of Pure Cell Populations Enables Unambiguous Genetic Analysis of Heterogeneous Formalin-Fixed Paraffin-Embedded Tumors by Next Generation Sequencing, *Scientific Reports* (2016). DOI: 10.1038/srep20944

Provided by Silicon Biosystems