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Single-cell sequencing leads to a new era of cancer research

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BGI, the world's largest genomics organization, developed single-cell genome sequencing technology and published two research papers for cancer single-cell sequencing in the research journal *Cell*. In the papers, which were published today in the same issue of *Cell*, BGI researchers applied their new single-cell sequencing (SCS) method to identify the genetic characteristics of essential thrombocythemia (ET, a kind of blood neoplasm) and clear cell renal cell carcinoma (ccRCC, a typical kidney cancer), and demonstrated that single cell analyses of highly heterogeneous tissues provide much clearer intratumoral genetic pictures and developmental history than previous bulk tissue sequencing.

The availability of BGI's SCS method opens new ways for the genetic study of tumors at single nucleotide resolution, especially for those where it is difficult to identify key mutations by previous bulk tissue sequencing. The single-nucleotide resolution of this method enables application to a variety of diseases and biological processes, such as studies on cellular heterogeneity of tissues, iPS or stem cells, pre-implantation genetic diagnosis and the genetic recombination of reproductive cells.

Cells are heterogeneous in multicellular organisms. The current high-throughput sequencing technology has been applied in a variety of fields of biological study, however, its obvious limitations on studying complex phenomena such as tumor evolution, early embryonic development, neuron science, and Meta genomics make it powerless on heterogeneous samples. Recently emerging single-cell analysis approaches like single-

nuclei sequencing on breast cancers by Navin et al. throw light on understanding the biology underlying cellular heterogeneity.

Until now, there has been no suitable way for scientists to explore the genetics of single tumor cells at a single-nucleotide resolution. To overcome this deficiency, researchers from BGI developed a high-throughput single-cell sequencing method based on an advanced multiple displacement amplification (MDA), and tested it using two single lymphoblastoid cells derived from a healthy individual (YH) who provided DNA for the first Asian diploid genome sequence. "Through the evaluation, we found our MDA-based method could provide greater resolution and genome coverage, which will enable single-cell analyses at a single-nucleotide level with relatively high sensitivity and specificity," said Luting Song, the leading author of this study and project manager at BGI.

BGI first applied its new SCS method to conduct single-cell exome analysis of the blood neoplasm because it is much more convenient to infer the development process underlying the abnormal proliferation of hematopoietic progenitor cells. Results revealed the JAK2-negative blood neoplasm may arise from monoclonal somatic mutant cells, and identified several known and novel mutated genes that may play roles in the blood neoplasm initiation and progression. Therefore, the identified mutated genes may be of interest for future biological research.

In addition, to better understand the intratumoral genetics underlying mutations of typical solid tumor, BGI researchers applied this new method to kidney tumor. The study demonstrates it is unlikely that this tumor resulted from two most common mutations in VHL and PBRM1. This emphasizes the importance of assessing and diagnosing cancers and patients at an individual level to determine the most effective treatment. Further analysis indicated that this tumor did not contain any significant clonal subpopulation. Quantification analysis of tumor heterogeneity

showed that most of the somatic mutations occurred only in a small fraction of the cells, and that mutations with different allele frequencies showed very different mutation spectrums. Researchers also screened for mutations in a group of 98 kidney tumor patients and identified potential key genes contributing to the establishment of this kidney tumor.

"Our pilot study demonstrates kidney tumor may be more genetically complex than previously thought and provides novel information that can lead to new ways to investigate individual tumors with the aim of developing more effective cellular targeted therapies," said Xun Xu, Vice Director of BGI. "This study also provides a good example of how single-cell exome sequencing could yield novel biological insights for an individual solid tumor."

"Our two studies demonstrate the power of our proprietary method for identifying complex, small genetic changes in a heterogeneous tumor at a greater resolution," said Yingrui Li, Vice Director of BGI. "I believe our study will enable researchers to develop new methods to clinically evaluate tumors and promote the research of complex diseases and biological processes."

Jun Wang, Executive Director of BGI, said, "BGI's single-cell sequencing technology elevates genomic studies to a new level, enabling researchers to conduct biological studies at the cellular level in life processes such as the growth, reproduction and development, heredity and aberrance of organisms."

Provided by BGI Shenzhen