single cell technology in tracing haematopoietic stem cell formation

Abstract: Haematopoietic stem cells (HSCs) is one of the most important stem cells in stems cells study. The molecular mechanism of its differentiation can help people understand many mechanisms of disease. Study on the single cell level can give a relatively more accurate data than the study on the population level. This review will introduce the single cell technology and show you a process to trace haematopoietic stem cell formation at single-cell resolution.

Introduction: Haematopoietic stem cells (HSCs) have long-term self-renewal potential and the ability to differentiate into various types of mature blood cells. We have been doing the longest and most in-depth study of the history of HSCs, which makes important guiding significance to the study of various types of stem cells, including cancer stem cells. Due to limitations separation and purification methods, researchers were only able to study the stem cells mostly based on the knowledge level of the population level. But the molecular mechanism of cell differentiation and many dizease happen on single cell level. In these cases, single cell technology is vitial to provide relatively more scientific and accurately data to the molecular mechanism.

In general, single cell technology include isolating single cells, analysis of single-cell cloning, real-time PCR of single cell and single-cell sequencing technology. There are three ways to isolating single cells-- limiting dilution, fluorescence-activated cell sorting flow (FACS) and micromanipulation, of which the most important way is fluorescence-activated cell sorting flow. Limiting dilution method is mainly through a series of dilution and eventually obtain a single cell. This way is very traditional, but is widely used as well. It is mainly used in different tissues of stem cells colony formation in vitro analysis. FACS is to use the flow cytometry,marking the cell surface markers or characteristics by means of specific groups of cells to obtain a single cell or a little population of cells. Micromanipulator is to use the micromanipulator microscope visually isolated single cell. It is mainly applied when the cell population is small and not suitable for FACS. Real-time single cell PCR is to design highly specific probes, PCR instrument using a single cell at the single cell level to detect a variety of high-throughput gene expression technology. In this way , we can get semi-quantitative detection result of the expression level of DNA and RNA in a single cell. Single-cell sequencing technology includes single-cell genome sequencing and single cell transcriptome sequencing.

In this experiment, the researchers devide the whole experiment into six parts.

First, isolate single T1 pre-HSC by CD201. In this part, the researcher used co-cultures seeded in the way of three cells and single cells, and then transplantation. The result shows that the HSCs from three primary repopu-lated recipients receiving single-cell-initiated cultures got the self-renewal ability.

Secondly, the researcher identify the T2 by single-cell-RNA-seq. researcher prepared 5 related populations , which are in the E11 AGM region, (1) ECs, CD31+VE-cadherin+CD41−CD43−CD45−Ter119−; (2) T1 preHSCs, CD31+CD45−CD41lowc-Kit+CD201high; (3) T2 CD41low, CD31+CD45+CD41low; and in fetal liver, (4) E12 HSCs, Lin−Sca-1+Mac-1lowCD201+; and (5) E14 HSCs.[1] The five groups each performed ten cells and single cell test in the same enviorment. The test result shows that the T2 CD41low population can be divided into two distinct subpopulations. And subpopulation A and T1 pre-HSC. has the similar gene expression profiles. But for several molecules, they havevery different expression. Subpopulation A is always expressing some EC markers and HSC-related transcription factors. Subpopulation B had myeloid lineage signatures. The heterogeneity T2 CD41low population shows in the result can lead to the suggestion that myeloid cell contamination in the presumed T2 pre-HSCs.[2] The following experiment also confirm this point.

Thirdly, the researcher tested the global gene expression dynamics in the HSC.

In this section, the researcher used three clusters-- ECs, pre-HSCs, and mature HSCs. Using PCA to get the amount of mRNA in the cell to anlyse the expression of DNA.,and then use Gene ontology (GO) analysis to know the enriched terms. In this experiment ,they analysed 324 genes from the blood-vessel-related GO terms. Most of genes that were differentially expressed were those consistently downregulated from ECs to HSCs. This fact to a degree showed that pre-HSCs maybe have a less intimate lineage relationship with venous ECs than with arterial ECs. Scientist thinks that ECs are located in major arteries and never give rise to HSCs in veins. But recently, studies have shown that arterial ECs and HSCs are originated from distinct precursors. [3]. This experiment seems to give a evidence that the pre-HSCs has a nearer relationship with arte-rial ECs.

The next step is to analyze the specific role of mTORC2 signaling. Compare the gene set enrichment analysis (GSEA) with Kyoto Encyclopedia of Genes and Genomes (KEGG) to research that weather the signaling path-ways is involved in HSC formation. In this process, it shows that the mTOR signaling pathway has a higher level in T1 pre-HSCs than ECs. Another finding is that rictor was indispensable for HSC emergence from endothelial cells, but was required less in later haematopoietic progression during development.

Then the reserchers analyzed the dynamic transcription factor and surface marker

Expression.

The last part is to analyze the signature genes and cell-cycle feature.

Through this six sections of the experiments, the researchers use the single cell technology to trace haematopoietic stem cell formation at single-cell resolution. This is the first time that trace the process in the single –cell level. This experiment provide nice data about the molecular mechanism in the haematopoietic stem cell formation.

Single cell teachnology will become an important way to analyse the cell life process. In this experiment, the researcher set different controlled trials to analyze different HSC. Those controlled trials are very delicate. And this investigation are the fundamental to reveal the novel mechanisms for HSC and provide a good example to other research.

Reference:

[1] Tracing haematopoietic stem cell formation at single-cell resolution *Nature (2016) doi:10.1038/nature17997* Fan Zhou, Xianlong Li, Weili Wang, Ping Zhu, Jie Zhou, Wenyan He, Meng Ding, Fuyin Xiong, Xiaona Zheng, Zhuan Li, Yanli Ni, Xiaohuan Mu, Lu Wen, Tao Cheng, Yu Lan, Weiping Yuan, Fuchou Tang& Bing Liu.

[2] Applications of Single Cell Technologies in Stem Cell Research Chinese Journal of Cell Biology, Vol 35, page 86-91. Dong Fang; Yuan Weiping; Cheng Tao.