1 Can the Single Cell Make Biomedicine Different?

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Abstract

The single-cell as the basic unit of biological organs and tissues has recently been considered an important window to furthermore understand molecular mechanisms of organ function and biology. The current issue with a special focus on single cell biomedicine is the first effort to collect the evidence of disease-associated single cell research, define the significance of single cell biomedicine in the pathogenesis of diseases, value the correlation of single cell gene sequencing with disease-specific biomarkers, and monitor the dynamics of RNA processes and responses to microenvironmental changes and drug resistances.

Keywords

Single cell · Sequencing · Imaging · Bioinformatics · Systems biology

1.1 Introduction

The single-cell as the basic unit of biological organs and tissues has recently been considered an important window to furthermore understand molecular mechanisms of organ function and biology. With the development of single cell biotechnology, the single cell biomedicine becomes more and more important area to understand the heterogeneity among cells, identify disease-specific biomarkers, and explore molecular regulations and signals. The single cell systems biology is emphasized as an approach to understand single-cell mechanical phenotypes, single-cell biology, heterogeneity and organization of genome function [1]. Multi-dimensional, multi-layer, multi-crossing and stereoscopic single-cell biology definitely will benefit the discovery and development of disease-specific biomarkers, translation of single-cell systems biology into clinical phenotype, and understanding of single-cell gene sequencing and function in patient response to therapies. As a part of single cell biomedicine, single cell RNA sequencing (scRNA-seq) is used as a critical and initial tools to define the alterations of transcriptomes, development of intratumor and intercellular heterogeneity, and genotoxicity in response to drugs [2]. scRNA-seq can detect somatic mutations and epigenetic alterations in evolution, post-transcriptional RNA modifications, and RNA editing. It is also important to illuminate the effects of single-cell RNA

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isoform diversity on gene, protein expression, and regulation. The current issue with a special focus on single cell biomedicine is the first effort to collect the evidence of disease-associated single cell research, define the significance of single cell biomedicine in the pathogenesis of diseases, value the correlation of single cell gene sequencing with disease-specific biomarkers, and monitor the dynamics of RNA processes and responses to microenvironmental changes and drug resistances.

1.2 Rapid Development of Single Cell Measurements

The accuracy of targeted single cell isolation, purification, and measurement is the critical step to ensure the trust of single cell biomedicine. Tatematsu and Kuroda as the leading scientists developed an automated robot that facilitates non-invasive isolation of a single cell with the most favorable properties from arrays containing $>10^5$ cells, and described the system as a "single-cell robot" to compare with a conventional fluorescence-activated cell sorter [3]. Such system can carry out a high-throughput screening for single cell isolation with targeted labelling and perform the comprehensive analysis the biological function of receptor-associated signaling between single cells. In addition, they also clearly described the advantages between single colony-based and single cell-based breeding methods, and between "single cell robot" with conventional single-cell analysis, automated single-cell analysis, or other cell screening methods enabled by automated single-cell analysis. It is more critical to monitor alterations of single-cell dynamic phenotypes during evolution, microenvironmental changes, disease progression, and therapy, with the development of single-cell technologies in the deep understanding and value of the constituents within dynamic phenotypes [4]. The detection of single-cell dynamic phenotypes will require more precise readouts from "single cell robot", not only on the findings but also the meaning of tumor heterogeneity and evolution to carcinogenicity, metastasis, and responses to targeted therapies.

Huang et al. systemically addressed the methodology of high throughput single cell RNA sequencing, bioinformatics analysis and applications, by combining their own innovative experience in the current volume [5]. Hou's group as one of the pioneer scientists on the development of high throughput scRNA-seq published their early work to measure the single-cell exome sequencing on a clear cell renal cell carcinoma and its adjacent kidney tissue to better understand the intratumoral genetics underlying mutations of cancer in 2012 [6]. It was proposed that such quantitative population genetic analysis as new ways could identify the clonal subpopulations, mutation spectrums, or detailed intratumoral genetic landscape at a single-cell level. They furthermore described the difference of large-scale scRNA-seq library preparations in the accuracy and throughput of scRNA-seq, and the importance of computational analysis of scRNA-seq data. When analyzing scRNA-seq data, we should clearly define what the quality control, criteria of subpopulations identified, or differential expression and transcript isoforms across conditions are, how expression estimation and normalization, pseudotemporal ordering, or interrogation of spatial information are settled out, as well as why network inference, differential Splicing, or allelic expression patterns should have the special attentions.

More approaches are used to determine dynamic phenotypes of single cells by the cross-disciplinary nature of these techniques, e.g. quantitative live cell imaging, time series analysis, computational modeling, and statistical testing on multi-dimensional data sets. Ruderman headlined the computational models as a predictor reflecting the quantitative phenotypes of cells, new theories as a system screening the key response variables of phenotypes, or multidisciplinary dynamic phenotype research teams [7]. Feng et al. overviewed and discussed recent applications of super-resolution techniques in single cell imaging for multi-dimensional, multi-color, live-cell imaging [8]. The quality and potentials of multimodal imaging are compared

among stimulated emission depletion, structured illumination, single-molecule localization, and other super-resolution microscopies. It will significantly improve our vision and understanding of single cells if cell imaging can be integrated with molecular biology, signaling, regulation, and bio-computing algorithms.

1.3 Single Cell Biology in Development and Evolution

Single-cell transcription kinetics and variability play an important role in cell development and evolution through gene regulation. Of those, noncoding RNA (ncRNA) is suggested to regulate cell mechanic changes and volume flexibility. Fu et al. illuminated the emerging single-cell RNA sequencing technique and he expression of ncRNAs during embryo development [9]. The expression of ncRNAs within single cells measured with single-cell RNA-seq techniques can vary with stages of embryonic development. ncRNA, especially lncRNAs and miRNAs, can regulate and prevent embryonic cell development from the disorder. Although partial functions of single-cell lncRNAs and miRNAs was explored, the most of single-cell circRNAs, piRNAs, or snoRNAs functions remain unclear in embryonic development. Wei et al. described the contributions of single cell genetics and epigenetics in early embryo from basic research to reproductive medical application and the knowledge of programming/reprogramming and the epigenetics dynamics in the cell lineage differentiation [10]. This is a special vision from the reproductive medicine to evaluate the meaning of embryo or polar body scRNA-seq to genetic diagnosis and prediction. The single cell techniques and bioinformatics analyses for early embryo were listed and compared with other tissue cells. Single-cell biomedicine in the development will provide the details of each cell origination and sources as well as molecular mechanisms by the landscape shaped itself. Single cell DNA methylation will demonstrate the mechanisms of cell lineage differentiation, gene expression heterogeneity in the pluripotent state of mouse embryonic stem cells, or the start of a lineage transition or a transient phase of altered sensitivity to lineage-specific signals.

1.4 Heterogeneity of Single Circulating Tumor Cells

The cancer heterogeneity can be described by single cell sequencing and comprehensive molecular characterizations of cancer cells, including hereditary and somatic gene changes and mutations. The specificity, characterization, and roles of cancer cell heterogeneity can decide the sensitivity and resistance of cells to therapies and be considered as the critical factor to develop target-driven therapies and strategies applied in clinical trials based on a proposed precise self-validation system [11]. Cancer heterogeneity can act as a potential cause of drug resistance to targeted therapy, contribute to tumor evolution and adaptation, and influence the efficacy of personalized-medicine strategies. The influence of tumor heterogeneity on drug efficacy and resistance should be monitored by disease- and biology-specific biomarkers [12]. The intelligent single-cell robot of human cells were proposed to integrate together systems information of molecules, genes, proteins, organelles, membranes, architectures, signals, and functions to assist clinicians in the decision-making, molecular understanding, risk analyzing, and prognosis predicting [13]. Heymann and Téllez-Gabriel pointed out the characterization of heterogeneity among circulating tumor cells (CTCs) at the single cell level could be an important approach to explore the causes and progression of disease and the accurate selection of molecular biomarkers [14]. This is an initiative of disease-orientated figure to enrich, isolate, purify, and measure the single CTCs at different levels, including RNA, DNA, protein and epigenetic events. In addition to the value of cancer indication, the single CSCs will provide more clinical and biological importance to identify the heterogeneity, origin, subtypes, and malignancy of the cancer. The single circulating cell will be the major source and play the

critical role in identification and validation of disease-specific biomarkers metastases, drug resistance, prognosis, phenotypes, metabolism, or proliferation. With advances in single-cell sequencing technologies, the complete genome of the single CTC can be defined and compared with corresponding primary and metastatic tumor single cells to monitor genomic variations in metastatic and recurrent tumors, infer tumor evolution during treatment, and examine mechanisms of the epithelial-mesenchymal transition [15]. The sequencing of single CTC genomes and transcriptomes is even more complex and difficult, e.g. eliminating backgrounds of white blood cells, isolating and collecting cells without damaging or losing DNA and RNA, obtaining unbiased and even whole-genome and transcriptome amplification material, and analyzing sequencing data.

1.5 Single Cell Values in Cancer

The cancer is a major area where the single cell technologies were applied mostly to define the heterogeneity of intra- or inter-tumor cells, rare cancer cell types, gene mutation and characters, evolution and developmental lineage relationships, or sensitivity to therapies. Lung cancer is one of the most severe cancers with the highest incidence and mortality, with a complex mechanisms and available targeted therapies. A large number of lung cancer-associated biomarkers have been developed to monitor the severity, duration, subtypes, and transit from chronic lung diseases to cancer [16–19]. Wang and Zhang brought out single cell proteomics as a front point of single cell biomedicine with a clear focus on lung cancer and summarized potential technologies to measure single cell protein profiles [20], including flow cytometry, mass cytometry, microfluidics and chip technologies, chemical cytometry, single-cell western blotting, or quantity and functions of proteins. The single-cell proteomics are mainly applied for the identification and screening of diagnostic biomarkers and therapeutic targets for prevention, early detection, prognosis, and response to therapy, as well as for the understanding of mechanisms. While, the single cell sequencing is often used to identify gene mutations and intercellular heterogeneity. It would be important to define the correlation and biological consequences between the gene mutation and protein expression at the single cell. As Yu et al. summarized in the current book [21], the single-cell sequencing has been widely applied in cancer research, e.g. breast cancer, ovarian cancer, lung cancer, hematopoietic tumors, renal cell cancer, glioblastoma, circulating tumor cells, or cancer stem cells. In addition to the gene mutation, the single cell sequencing is expected to provide more indications or potential evidence on which clinicians can consider or select the individualized or targeting therapies. The single cell sequencing can benefit to identify the new sub-populations of cancer cells, the variation between cancer cells and cancer stem cells, and the development of drug resistance.

Hematological malignancies are one of challenging cancers with poor prognosis and non-specific therapies due to the downregulation of target antigens and the immunosuppressive environment against the host immune response [22]. A number of potential immunotherapies, e.g. T cells, NK cells, or monoclonal antibodies, or inducing and/or recovering T cell activation, provide the exciting future for the patients, while the large number of blood cancer cell heterogeneity as an important factor in response to treatment may influence or decline the efficacy of therapies. Chu et al. recently emphasized there is a great heterogeneity among subclones and their extensions, especially in hematological malignancies and called special attention to define the aggregate populations, intra-clonal and inter-clonal heterogeneity, and its frequency, using single cell sequencing [23]. It seems that single cell systems biology may generate more unique and important information on cancer cell subtypes, heterogeneities, or epigenetics to assist clinicians in the diagnosis and therapeutic design for diseases and in the prognosis of patients with individualized therapies. Shi et al. furthermore overviewed potential roles of single cell sequencing in the diagnosis and treatment of hematologic malignancies and tried to headline the advantages

of the single cell biology from the clinical point [24]. This particular article collected the scientific evidence from studies and summarized genomic, transcriptomic, and epigenomic findings in single cells of acute leukemia, multiple myeloma, or chronic myeloid leukemia. The most valuable point of single cell biology, e.g. circulating tumor cell sequencing, is to monitor minimal residual disease of hematologic malignancies and define functional heterogeneity and clonal evolution in such life-threatening hematological diseases.

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas (CRISPR associated) system has been applied in many aspects to understand the molecular mechanisms of a gene, signal pathway, and regulatory function, as "the solution for gene editing's Gordian knot" [25]. For example, CRISPR was proposed to play the important role in the understanding of drug genotoxicity and resistance, during which how gene changes, mutations, and heterogeneity may control and dominate the cell signaling, regulation, and sensitivity to drugs [26]. During the interaction between cells and drugs, the perturbation and phenotype of a cell can be changed and monitored using the single-cell CRISPR screening. In addition, CRISPR can be one of the most important genome editing-assisted gene knock-in technologies, to repair genetic changes and cure inherit diseases [27]. In the current volume, Qian and Wang demonstrated that the RNA editing as a RNA structure research tool also plays important role in cancer research, especially in the understanding of biological function of RNA species, structures, and expression [28].

Nowadays, transcriptomics studies mainly focus on three aspects, the RNA species (mRNA and non-coding RNA), the RNA structure (start sites, splicing patterns and post-transcriptional process) and the expression levels of RNA. Among them, the RNA structure research tool, RNA editing, remains the least popular one which we still have more to explore on the role of it in cancer research [28]. RNA editing enzymes such as ADARs and APOBECs are all promising targets in cancer therapeutic strategy. Here we listed several examples of RNA editing studies in some cancers. However, their pathways are differentially regulated in cancers which should be further clearly studied. The best tool to study RNA editing is NGS. Here, we also discussed the challenges and the possible ways to overcome them. We are sure to believe that RNA editing performed by NGS has the ability in studying transcriptomes, even at single cell level. It will be sure to help a lot in cancer diagnosis and treatment in the near future.

1.6 Other Biological Significance

Single-cell-based biotechnologies can be also used in multiple aspects. Voigt A et al. initially proposed to develop protein-based therapies, e.g. antigen-specific monoantibody, through single cell system [29]. The specificity of protein-based therapy can be screened and validated in the single cell system. Single cell analysis can define the heterogeneity-associated efficacy among cells. The measurement of such heterogeneity is fully dependent upon the quantitative accuracy of scRNA-seq, including the protocol, RNA reverse transcription, or cDNA pre-amplification [30]. In addition, scRNA-seq is suggested as a powerful tool to measure the heterogeneity and germline of stem cells [31]. Furthermore, scRNA-seq was applied in pulmonary epithelial cells isolated and harvested from the lung of animals or patients suffered from diseases [32].

In conclusion, the current issue with a special focus on single cell biomedicine is the first effort to collect the evidence of disease-associated single cell research, define the significance of single cell biomedicine in the pathogenesis of diseases, value the correlation of single cell gene sequencing with disease-specific biomarkers, and monitor the dynamics of RNA processes and responses to microenvironmental changes and drug resistances.

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