Understanding Diseases from Single-Cell Sequencing and Methylation

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Abstract

Clinical single-cell biomedicine has become a new emerging discipline, which integrates single-cell RNA and DNA sequencing, proteomics, and functions with clinical phenomes, therapeutic responses, and prognosis. It is of great value to discover disease-, phenome-, and therapy-specific diagnostic biomarkers and therapeutic targets on the basis of the principle of clinical single-cell biomedicine. This book reviews the roles of single-cell sequencing and methylation in diseases and explores disease-specific alterations of single-cell sequencing and methylation, especially focusing on potential applications of methodologies on human single-cell sequencing and methylation, on potential correlations between those changes with pulmonary diseases, and on potential roles of signaling pathways that cause heterogeneous cellular responses during treatment. This book also emphasizes the importance of methodologies in clinical practice and application, the potential of perspectives, challenges and solutions, and the significance of single-cell preparation standardization. Alterations of DNA and RNA methylation, demethylation in lung diseases, and a deep knowledge about the regulation and function of target gene methylation for diagnosing and treating diseases at the early stage are also provided. Importantly, this book aims to apply the measurement of single-cell sequencing and methylation for clinical diagnosis and treatment and to understand clinical values of those parameters and to headline and foresee the potential values of the application of single-cell sequencing in non-cancer diseases.

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Keywords

Clinical single-cell biomedicine · Single-cell sequencing and methylation · Pulmonary diseases · Methodologies · Clinical diagnosis and treatment

With the rapid development of single-cell biology and sequencing, clinical single-cell biomedicine is defined a new merging discipline to integrate single-cell RNA and DNA sequencing, proteomics, and functions with clinical phenomes, responses to therapies, and prognosis. Several hospitals start a new independent practice to perform clinical single-cell biomedicine, although there are still many challenges to be faced and solved. Clinical single-cell biomedicine is more expected to dynamically monitor cell–cell variations and communications, drug efficacy and resistances, discovery and development of therapeutic targets, and genealogic phenotypes of cells during disease progression [1–3]. Clinical single-cell biomedicine will analyze inter- and intra-cellular heterogeneity, new cell category, dysfunctional regulatory networks, microbes, and disease evolution. In addition to understanding molecular mechanisms using single-cell sequencing and measurements, it is more important to discover disease-, phenome-, and therapy-specific diagnostic biomarkers and therapeutic targets on the basis of the principle of clinical single-cell biomedicine. As the part of clinical single-cell biomedicine, we demonstrated important roles of single-cell sequencing in systems immunology in our previous book entitled "Single Cell Sequencing and Systems Immunology" [4], e.g., as a tool to deeply understanding the development and regulation of systems immunology. In this book, we furthermore overviewed the roles of single-cell sequencing and methylation in diseases and explored disease-specific alterations of single-cell sequencing and methylation. This book specially focuses on potential applications of methodologies on human single-cell sequencing and methylation, on potential correlations between those changes with pulmonary diseases, e.g., lung cancer, chronic lung diseases, and allergic lung diseases, and on potential roles of signaling pathways that cause heterogeneous cellular responses during treatment.

The first part of the book emphasizes the importance of methodologies in clinical practice and application, the potential of perspectives, challenges and solutions, and the significance of single-cell preparation standardization. Pensold and Zimmer-Bensch [5] headlined the importance of accurate and reliable cell capturing in single-cell sequencing, overviewed the current state of single-cell isolation methods, and addressed key parameters like sample compatibility, viability, purity, throughput, and isolation efficiency. Gupta et al. [6] systematically described the value of single-cell sequencing in the investigation of T cell receptors and their transcriptional profiles and firstly prospected the importance of the technological development in translational and clinical application. This is an example to apply the single-cell sequencing for special target clusters in a special cell population and illustrate the translational strategy how the single-cell sequencing is developed for clinical application. The single-cell sequencing of T cell receptors has the great value to benefit immune-therapy for cancer and autoimmune diseases.

The methylation and demethylation of cytosine in promoter regions play an important role in the control and regulation of gene expression by the modulation of translation by modifying tRNA-bases or silencing. The process of the methylation within cells can be influenced by their environment or for the development of complex organisms, especially for organs/tissues which are exposed and connected directly to the environment, e.g., lung. This book discusses alterations of DNA and RNA methylation and demethylation in lung diseases and provides the deep knowledge about the regulation and function of target gene methylation for diagnosing and treating diseases at the early stage. Zhou et al. [7] demonstrated global methylation pattern and specific gene methylation status of associated genes in the development of pulmonary fibrosis and methylation patterns and severities of the promoter regions of Thy-1, COX-2, p14ARF, and PTGER2 genes as disease-specific biomarkers to

predict the occurrence and development of the disease. Using bioinformatics, Liu et al. [8] addressed that altered methylations of inflammatory cells downregulated the gene expression of inflammatory mediators and initiated the occurrence of lung diseases. The combination of expression quantitative trait loci and genome-wide association studies was suggested as a new strategy to identify alterations of target gene methylation in chronic lung diseases, e.g., lung fibrosis [9] or chronic obstructive pulmonary diseases [10]. Of many target genes, DNA methylation of RAS-association domain family 1 was proposed as a lung cancer biomarker for new therapeutic strategies and for monitoring the reliability and sensitivity of DNA methylation [11].

One of the important issues in this book is to apply the measurement of single-cell sequencing and methylation for clinical diagnosis and treatment and to understand clinical values of those parameters. Wu et al. clearly reported the urgent need to optimize and standardize the workflow and protocol as well as standard operation performance, the comprehensive single-cell database and knowledgebase, and the design of clinical studies among various hospitals during clinical application [12]. The importance of target gene methylation and expression phenomes, e.g., Aplasia Ras homologue member I [13], P16 gene [14], and related molecular mechanisms of tumorigenesis and progression in various types of cancers, is obvious. Of those, single-cell RNA sequencing can be utilized to identify subtypes of pancreatic cancer [15] and genitourinary malignancies [16] and to improve the quality, efficiency, and specificity of cancer diagnostics [17]. In addition, new therapeutic targets and strategies can be discovered and developed with the improvement of methodologies and knowledge on single-cell sequencing and methylation. Duncan et al. offered an example of PI3K inhibitors and a frontline view of biological effects of the PI3K pathway and multiple isoforms of PI3K, mutations found in the PI3K isoforms in many different types of cancer, and new strategy of combination therapies between PI3K inhibitors and other target-driven therapies [18].

One of advances in this book is to headline and foresee the potential values of the application of single-cell sequencing in non-cancer diseases, which will be the frontline science and need more efforts to be explored. Garcia et al. provided the comprehensive understanding of single-cell RNA sequencing in human renal, pancreatic, and viral diseases [19]. This is an important and expecting review to discuss the specific application of single-cell sequencing in cellular compositions, heterogeneity and uncovering clues of viral infections and diseases of the kidney and pancreas for the development of targeted and personalized therapies. Singh specially emphasized the importance of single-cell sequencing in the discovery of the drug resistance clone, intercellular variation and communication, mutations and transcriptional profiles of a pathogen across different stages of human genital infections [20]. Rajan and Dall'Acqua addressed the potentials of those advanced technologies in the discovery and development of antibody-based humanized therapies [21]. Single B cell sequencing will provide a new approach and emerging strategy for antibody-based therapy. Chang et al. summarized the potential application and values of single-cell sequencing in the development of neurological cells and microglia as well as single-cell changes during brain injury [22].

This book is one of initiatives to deeply understand the importance and value of single-cell sequencing and methylation measurement for clinical application, although there are still many challenges and obstacles to be broken through. It is also highly expected to translate the simultaneous measurement of both single-cell sequencing and methylation in a human cell, e.g., parallel single-cell genome-wide methylome and transcriptome sequencing. There is a rapid growth in the development and improvement of single-cell methylation and sequencing, e.g., single-cell bisulfite sequencing for genome-wide base-resolution mapping of single-cell DNA methylation, random displacement amplification sequencing for the first full-length single-cell RNA-sequencing method, single-cell and single-base resolution DNA methylation analysis based on reduced-representation bisulfite sequencing,

and single-cell, locus-specific bisulfite sequencing for cell-to-cell variability and the pathogenic history. Complete DNA CpG methylomes at the single cell can be screened and compared comprehensively through whole genome bisulfite sequencing, reduced-representation bisulfite sequencing, and enrichment-based methods such as MeDIP-seq, MBD-seq, and MRE-seq. At the end, we as co-editors of this special book would like to take this special opportunity to deeply appreciate all authors and contributors for the intensive and hard works to make this book possible for publication. We are especially grateful for those experts to review and comment chapters in order to maintain the high quality and look forward to working with all of you in future.

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