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Facts, Challenges, Difficulties and Hopes in Single-Cell Biology: Physiopathological Studies

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Abstract

Single-cell approaches are being increasingly used to unravel the many diverse mechanisms underlying biological processes that characterize each cell irrespective of the influx of other cells even within the same tissue. Consequently, the interference of metabolites and nervous stimuli emanating from the circulatory or nervous system in a higher organism like man is avoided. However, while the single-cell approach yields a wealth of data about single-cell metabolism and internal regulatory mechanisms, information about interactions and interrelations among similar or dissimilar cells may remain obscure. Starting from these considerations, here we summarize, without attempting to be exhaustive, some areas in which we think single-cell biological studies could be effective in translational medicine and in other areas of applied sciences. In this short review we describe the facts, challenges and perspectives related to these issues.

Keywords: Single cells; Single cancer cells; Pharmacology in single cells; Preimplantation genetic diagnosis; Preimplantation genetic screening

Commentary

Here we briefly recapitulate concepts and experimental findings that can enhance our understanding of physiological phenomena and/or their variations that lead to disease. Furthermore, we mention, albeit with a reductionist approach, the areas where breakthroughs have been made and where challenges remain, and what we may reasonably expect the future to bring.

Studies conducted with single cancer cells have been particularly fruitful, mainly as regards the capacity of these cells to act as early harbingers of the disease or of tumor relapse as well as a model to understand cancer pathogenesis, spreading and metastatization [1-4]. Furthermore, nucleic acid sequencing is starting to shed light on the molecular features of single cancer cells. Based on this information one may dissect events that belong to a specific cell population or organ whether healthy or diseased [5]. This applies to the diverse fields of physiopathology [6], particularly those of nanobiology [7] and immunology [8], and during development and tissue mosaicism [9].

Facts and perspectives are now emerging in the field of the three-D structural architecture of the genome [10,11], and in the field of the electrical characterization of single cells [12]. The latter aspect is particularly important for studies conducted with nervous, muscle and cardiac tissues using, for instance, such sophisticated patch-clump, ion diffusion and the very recent microfluidic technique [13].

Also in the field of pharmacology, a single cell can be studied and measured *in vivo* and studies can be conducted on committed cells starting from stem cells [14]. Furthermore, studies based on pharmacokinetics and pharmacodynamics using different drugs can help to determine the exact cell target where the "bullet" will operate to modify an altered status to a possible normal status [15,16]. This approach will help to define the pathway through which a stem cell reaches the final cell-fate decision thereby overcoming the difficulty inherent in studies of a population of multiple-fate cells [17]. For example, heterogeneity is extensive in hematopoietic stem and progenitor cells as well as in multipotent progenitors [18,19]. Notably, the root hair plant model in a single cell is generating data, also through a developmental approach, that have relevant consequences for the overall biological equilibrium of our planet [20].

A field in which single-cell analysis is already having a great impact on human health is the field of genome biology and medicine. In fact, single-cell genomics is being used in preimplantation genetic diagnosis, not only for aneuploidy screening but also to avoid single-gene disorders [21], chromosomal abnormalities [22], and to prevent transmission of X-linked disorders [23]. Preimplantation genetic diagnosis is an important feature of human reproduction not only because of ethical considerations but also in preventing the transmission of severe inherited disease in affected families [24]. In this context, ethic problems such as sex selection also with regard to social sexing should be addressed with a very open-minded philosophical attitude [25]. In the table are reported the most important facts, challenges, difficulties and hopes that appear to be the most fruitful areas to pursue future research in single-cell biomedicine.

To sum up, we prospectively illustrate some examples that are closely related to the contents of this Short Review: (i) The application of single-cell analysis in the field of cancer is rapidly expanding and promises to shed light on how a single cell (mainly a single stem cell) can become metastatic or lead to cancer progression and invasion. Furthermore, in cancer biology, it would in principle be more effective to dissect biomarkers in single-cell analysis in order to detect not only the early appearance of the transformation process but the tissue types from which the primary cancer originated; (ii) Single-cell analysis

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	Facts		Challenges	Difficulties	Hopes	References
Cancer biology	• Bulk tissue genomic analysis provides key insights into cancer biology but does not provide information about spatio-temporal cell interactions and the genetic events occurring during tumor progression		• To define clonal heterogeneity in tumor • To detect mutations and perform gene-expression studies on 'cancer stem cells' • To identify circulating tumor cells originating from a primary tumor	• Isolation of single tumor cells thereby minimizing their loss and genomic degradation • Reduction of the noise generated during sampling due to multiple layers of cell heterogeneity in cancer	• To detect subclones of cancer cells in order to shed light on cell interactions, on the development and control of metastatic disease, drug resistance and disease progression • To detect parts of cell components (e.g., exosomes, microvesicles) as biomarkers or tools for pathological studies	1-5,26-29
Cell population within tissue	• Analysis of bulk tissue samples does not reveal cell-to-cell variations or rare cells that may play an important role in disease progression • Single-cell sequencing (SCS) provides detailed comprehensive data about individual cells	• Neurobiology	• Neurons are one of the most morphologically diverse cell populations • Single-cell RNA sequencing is a powerful unbiased approach with which to classify neurons based on their transcriptional profiles	• Single neuron RNA sequencing must be combined with other techniques (e.g., electrophysiology and microfluidics) to obtain a more comprehensive functional profile from a specific brain microenvironment	• SCS may be a novel approach with which to classify neuronal cell types and identify possible DNA diversity in neuronal cell populations	30-33
		• Immunology	• The immune system is constituted by a wide variety of cell types that work together in a highly integrated fashion to recognize and eliminate antigens	• The transcriptional heterogeneity within cell types in response to a variety of antigens	• Unbiased RNA SCS methods may be used to investigate heterogeneous transcriptional responses in immune cells after antigen activation to: 1) Analyze cells that show variable responses when stimulated under different conditions *in vitro*; 2) Identify multimodal gene expression patterns of differentially stimulated cells	8,34-36
		• Tissue mosaicism	• The traditional view of somatic tissues is that normal single cells have identical genomes. However, this dogma is beginning to be challenged by increasing evidence of genetic mosaicism in normal tissues that arises during normal development	• Most studies have analyzed bulk tissue samples. Therefore, controversy exists about the prevalence of mosaic mutations and whether they can simply be explained by technical error	• SCS methods may provide a novel approach with which to resolve cell-to-cell variations in normal tissues at an unprecedented genomic resolution	9,37,38
3D genome architecture	• The chromosome organization and its spatial nuclear organization are linked to the control of gene expression, DNA replication and repair • The 3D genomic organization is cell type-specific		• The hierarchical 3D chromosome organization must be studied to fully understand the transcriptional behaviour of the gene	• Chromosome conformation capture methods (Hi-C) enables one to assess the probabilistic chromosome conformation averaged from millions of cells, but not to relate the chromosome conformation to the single genome activity pattern	• The recently developed single cell Hi-C protocol assesses the spatial organization of the entire genome in an individual nucleus and may provide structural information about individual cell variability	10, 11, 39
Pharmacology	• To enhance drug discovery and development it is crucial to measure pharmacokinetics (PK) and pharmacodynamics (PD) *in vivo* at single cell level	• Single-cell PK measurements	• Traditional PK, which is based on the measurement of plasma concentration, does not provide information about the cellular heterogeneity of drug distribution	• The cellular location within tissue can modify the concentration time course of a drug and can significantly affect treatment response	1) To follow drug distribution and uptake at single-cell level using intravital microscopy. In fact, multichannel imaging may be used to identify different cell populations and to measure drug uptake within these cells *in vivo*; 2) Measurement of a drug concentration in different cell types within a tissue may be a means to understand and optimize local or targeted drug delivery	14-17
		• Single-cell PD measure-ments	• To establish drug efficacy one must measure and understand not just the drug-target engagement but also the downstream PD response	• The time-course of drug distribution and target interaction differs significantly at single-cell level	1) To evaluate the downstream PD response at single-cell level through intravital microscopy: fluorescent genetic reporters may allow the real-time measurement of relevant cellular pathways 2) To monitor the real-time distribution and single-cell uptake of drugs would provide an integrated PD/PK perspective	
Preimplantation genetic diagnosis	• Single gene mutation		• A comprehensive approach is required for the simultaneous detection of monogenic and chromosomal disorders	• DNA contamination •Single-cell based PCR artefacts: allele drop out, random failure of amplification of one allele at a locus, the richness of the locus in G/C bases, chimeric DNA molecules and copy error • Biologically variable noise: the cell-cycle stage of the isolated cell	• To develop a genome-wide method using next-generation sequencing for single-cell genome characterization to accurately determine genomic variations, such as copy number variations and single or small nucleotide variations simultaneously	21-25, 40-44
	• Chromosomal abnormality					
	• Mitochondrial DNA copy number					

Table: Facts, challenges, difficulties and hopes in single-cell biology.

of cell populations within a given tissue will reveal differences in the whole genome RNA transcriptome. The main cell differences that derive from gene expression analysis are pertinent not only to each cell type but above all to pathological aspects during a disease. These cells can also characterize a cell genomic mosaicism which raises additional questions; (iii) Pharmacological studies at both pharmacodynamic and pharmacokinetic level can be more precisely focused when single-cell analysis is characterized as illustrated in point i; (iv) Preimplantation genetic diagnosis could revolutionize the timing and hence the impact of molecular diagnosis and the efficiency of *in vitro* fertilization techniques. In fact, once the genome-wide method becomes optimized, it will be possible to evaluate a single gene disorder and chromosomal abnormalities simultaneously. In this context, next-generation sequencing may lead to the development of a genome-wide procedure for single-cell genomics.

In conclusion, we think that the era of single cell biology is still in its infancy and that the next few years will see unprecedented scientific progress along the lines prospected herein.

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