The Organoid Cell Atlas: A Rosetta Stone for Biomedical Discovery and Regenerative Therapy

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Keywords: Organoids, single-cell profiling, high-throughput imaging, multi-omics technology, data resources, bioinformatic tools, integrative analysis, biomedical applications

Main text

The case for an Organoid Cell Atlas

Human organoids – three-dimensional structures of cells that recapitulate important aspects of organ development *in vitro* – hold tremendous potential for biomedical applications[1-3] (**Fig. 1a-b**). First, organoids provide tractable models of human physiology and pathology, thereby enabling interventional studies that are difficult or impossible to conduct in human subjects. For example, organoids allow genetic and pharmacological manipulation in a complex cellular context that reflects human biology, and they make it possible to investigate the early stages of organ development and disease onset, which are hard to study *in vivo*. Second, organoids complement (and may eventually replace) animal models in many areas of preclinical drug development. Third, organoids provide patient-specific "avatars" for drug development and personalized therapies, including cancer therapy, rare genetic diseases such as cystic fibrosis, and complex, multifactorial disorders such as epilepsy. Fourth, organoid technology is expected to contribute to regenerative medicine, with the long-term goal of producing functional biological structures that can be transplanted into patients.

To realize the full potential of human organoid technologies, key challenges need to be addressed (**Fig. 1c**). Most immediately, we need better characterization and validation of organoids as faithful models of human biology, for example by defining quality standards for cell composition, cellular differentiation, cell states, and response to stimuli. A future catalog of well-characterized human organoids should include extensive replication as well as genetically diverse sample donors, in order to distinguish between biological and technical sources of variation, and to chart inter-individual variation in the human population.

Current organoid protocols still have relevant conceptual and technical limitations. For example, the resulting organoids do not faithfully represent the diversity of cell types in primary tissue (including non-parenchymal cells such as immune cells and stroma), nor do they incorporate the sustained effects that environmental exposures and organismal ageing have on human organs *in vivo*. It will be important to develop robust protocols that yield organoids with adequate tissue organization, differentiated cells, vascularization, immune cell infiltration, and for some organs (e.g. skin and intestine) even a microbiome. Moreover, we are still learning how to use organoids most effectively for discovering novel biology (e.g., through genetic and pharmacological perturbations) and how to exploit them for drug development and personalized medicine.

Single-cell omics and spatial profiling have a key role to play in addressing these issues (**Fig. 1d**). Comprehensive molecular maps of organoids and organoid development can reveal cell states and transcription-regulatory programs in unprecedented detail, and the comparison to corresponding human *in vivo* tissue provides powerful new ways of evaluating organoids. Single-cell epigenome/transcriptome profiling yields a quantitative, high-dimensional assessment of cell composition and cell states within organoids. Spatial profiling technologies characterize tissue organization and three-dimensional architecture. These methods are also useful for organoid quality control (e.g., identifying outliers, missing cell types, or aberrant gene regulation), and they can provide reference atlases for disease-centric studies. Furthermore, comparative molecular profiling of organoids and matched *ex vivo* tissue samples can guide the development of new and improved organoid protocols, for example by identifying missing stromal cell populations or relevant bottlenecks of cellular differentiation in organoids. Finally, single-cell technologies provide a powerful and scalable readout for functional experiments and for genetic/pharmacological perturbations that are tested in human organoids.

To help deliver on the promises of combining human organoids with single-cell technology, we have launched an Organoid Cell Atlas pilot project, as a 'Biological Network' within the Human Cell Atlas (HCA)[4-6], focusing on single-cell profiling of organoids and *in vitro* cell models (https://www.humancellatlas.org/coordinators/). The Organoid Cell Atlas will foster the production, quality control, dissemination, and utilization of single-cell and spatial genomics data for human organoids, and it will connect such datasets with the comprehensive profiles of primary tissue that are being generated within the HCA. A first step toward establishing the Organoid Cell Atlas has recently been funded by the European Union Horizon 2020 call for "Pilot actions to build the foundations of a human cell atlas" through the "HCA|Organoid" project (http://hca-organoid.eu/).

The Organoid Cell Atlas within the HCA

The Organoid Cell Atlas will be a cornerstone of the Human Cell Atlas (HCA)[4-6]. It is central to the HCA's mission to create comprehensive reference maps of all human cells – the fundamental units of life – as a basis for understanding human health and for diagnosing, monitoring, and treating disease.

The foundational goal of the HCA is to map and catalog cells, seeking to establish comprehensive, high-resolution maps of cell, tissues and organs in the human body. As it was the case with the Human Genome Project[7], the Human Cell Atlas is expected to create substantial biomedical impact, most notably in areas such

as precision medicine and regenerative biology. Human organoids are envisioned to play an essential role for the HCA[6], as they complement the profiling of primary tissue samples with a readily perturbable model for functional studies, while also benefitting from the profiling of human tissues to which they can be compared.

Some of the first applications of single-cell profiling to organoids included single-cell RNA-seq analysis of the cellular composition of mouse intestinal organoids[8] and human brain organoids[9]. Other pioneering studies uncovered complex cellular networks[10] and utilized single-cell profiles to improve organoid derivation protocols[11]. These studies reported remarkable parallels between organoid development, human primary tissue, and *in vivo* organogenesis, which reinforced the potential of organoids for dissecting human biology *in vitro*.

To foster synergies between single-cell profiling and organoid technologies, the Organoid Cell Atlas has been initiated in close coordination with the HCA leadership, following the example of other specialized atlases such as the Pediatric Cell Atlas[12] and the Human Tumor Cell Atlas Network[13]. The Organoid Cell Atlas is an open, collaborative network that seeks to: (i) encourage and standardize single-cell profiling of human organoids; (ii) facilitate access to single-cell organoid data (and informative metadata) via HCA data infrastructure; (iii) establish computational methods and tools for connecting organoid profiles with primary tissue data; (iv) put organoids into their biological context using HCA profiles of the *in vivo* counterparts. The envisioned data integration will be lightweight and synergistic, most notably driven by an Organoid Cell Atlas Portal that will build on and extend the existing HCA data infrastructure. Key features will include interactive data exploration, mapping between *in vivo* and *in vitro* data, and quantitative analysis of biological variation.

Establishing an initial version of the Organoid Cell Atlas

The EU H2020 HCA|Organoid project enables us to establish a first version of the Organoid Cell Atlas over the coming years, which may act as a nucleus for a broader, collaborative, global initiative. In this pilot project, we are generating single-cell transcriptome profiles, epigenome maps, and detailed imaging data in a selection of human organoids. For two organs (colon, brain), we derive and characterize organoids from 100 whole-genome-sequenced individuals each, in order to capture normal population variation and to establish a reference for disease-centric studies. We also develop an interactive, openly accessible, web-based computational platform – the Organoid Cell Atlas Portal (**Fig. 2a-b**). This portal will provide user-friendly access to single-cell data of organoids, and connect organoid profiles with single-cell data of human primary tissues.

We selected colon and brain organoids as the two focus areas of the HCA|Organoid project, for three reasons: (i) colon and brain were among the first organs for which organoids were demonstrated, such that relatively mature protocols are now available; (ii) colon organoids are derived from adult stem cells in primary *ex vivo* samples, while brain organoids are derived from pluripotent cells, thus spanning the two main sources of organoid derivation; (iii) colon and brain organoids have already been used for disease-centric studies, and single-cell characterization of these organoids for a large number of individuals will facilitate biomedical applications. Beyond the initial focus on colon and brain organoids, the HCA|Organoid project is designed such that most of the data infrastructure is generic and applicable to other types of human organoids. We actively seek collaboration with other projects that pursue systematic single-cell profiling in any type of human organoids, in order to explore the possibility of interconnection or integration with the Organoid Cell Atlas.

A central aim of the EU H2020 HCA|Organoid project is to develop an Organoid Cell Atlas Portal, which will be a computational infrastructure combined with a web-based frontend that makes single-cell data of human organoids easy to access and analyze. This effort will build upon the existing Data Coordination Platform infrastructure of the HCA (http://data.humancellatlas.org) for data submission, processing, annotation, and

retrieval. Key features that are specific to organoids will include the interactive exploration of human organoid data, data-driven selection of organoids for functional experiments, and comparison of disease-specific organoids against reference collections of normal organoids.

The Organoid Cell Atlas Portal will provide interactive mappings between single-cell profiles of human organoids and of the corresponding primary tissues available within the HCA, using algorithms that enable cell-cell alignments between these datasets. This functionality will facilitate and encourage the use of organoids as a model for detailed biological experiments, including the identification of candidates for molecular interventions. The mapping and data integration will also allow for exploring normal variation between individuals (e.g. due to common genetic differences) in an interactive manner, leveraging organoids as a model for the corresponding variation in primary tissues. Finally, the cell-cell alignments will contribute to analyzing and interpreting perturbations in human organoids in the context of the corresponding primary tissues.

We pursue several complementary strategies to ensure that the data in the Organoid Cell Atlas will be of the highest possible quality and reproducibility. First, we will invest in standardization and validation of experimental workflows for organoid derivation, by comparing protocols and assessing the relative contribution between biological and technical factors to the single-cell profiles of colon and brain organoids. Second, we will contribute to HCA efforts that establish community standards and software infrastructure for data processing and data annotation, for example by ensuring compatibility with the specific metadata structure of organoids. Third, we will develop and validate computational methods for the flexible alignment and comparison of cells between organoids and corresponding primary tissue. Fourth, we will implement interactive visualization tools that enable user-friendly quality control and exploratory analysis of new single-cell datasets of organoids contributed to the Organoid Cell Atlas.

To maximize the utility and impact of the EU H2020 HCA|Organoid project for the broader scientific community, we are pursuing a four-pronged strategy. First, the newly established organoids will be made available as a "living biobank" via Hubrecht Organoid Technology[14] (colon) or as a set of precise protocols for derivation from biobanked iPS cell lines (brain). Second, single-cell profiles will be made public as rapidly as possible, in concordance with the HCA's strong commitment to data sharing, local ethical regulations, and the European data protection law (GDPR). Third, we will evaluate and explore the practical utility of the Organoid Cell Atlas in a series of disease-centric pilot studies, pursuing CRISPR single-cell profiling using CROP-seq technology[15] and disease modeling of genetic epilepsy using brain organoids selected from the Organoid Cell Atlas. Fourth, we are committed to developing the Organoid Cell Atlas Portal into a public, sustainable, and widely used infrastructure for finding, accessing, analyzing, and interpreting single-cell data of human organoids.

Future directions and anticipated impact of the Organoid Cell Atlas

The combination of organoid technology and single-cell profiling creates a unique opportunity for advancing biomedical research and innovation. Enabled by recent technological advances, the human is becoming the leading "model organism" for the discovery of important new biology and the development of novel therapeutic strategies. The HCA is catalyzing this paradigm shift by establishing reference maps of all human cells, for the first time providing a detailed molecular picture of the human body. However, it is important to emphasize that therapy means intervention, and molecular mechanisms require validation. To deliver on the HCA's goal of deeply impacting biology and medicine, faithful *in vitro* models such as organoids are urgently needed, in order to enable detailed functional dissection and perturbation studies in a human context.

The Organoid Cell Atlas seeks to establish organoids as *in vitro* models within the HCA, kickstarting a collaborative effort to map the connections between cell types observed *in vivo* and their counterparts in organoids. This will guide the development of new organoid models that recapitulate the heterogeneous complexity of primary tissues, and it will leverage the power of organoids for large-scale perturbations and functional studies in human. The EU H2020 HCA|Organoid project is committed to establishing a practically useful and readily extendible initial version of the Organoid Cell Atlas within two years, while extensive further work will be needed to maximize the impact of the Organoid Cell Atlas for basic biology and biomedical applications.

The ultimate goal of the Organoid Cell Atlas is to help advance biomedical discovery and the development of regenerative therapies, for example by supporting disease-centric research in areas such as rare genetic diseases, complex multifactorial diseases, and precision oncology. This long-term goal can be achieved by creating an open and inclusive research environment that facilitates collaboration among a broad range of interested researchers, bridging communities and integrating expertise in organoids and single-cell technology.

Acknowledgments

We would like to thank Sabrina Ladstätter for comments on the manuscript and Bobby Rajesh Malhotra for assistance with figure preparation. The HCA|Organoid project is funded by the European Union's Horizon 2020 research and innovation programme (grant agreement n° 874769, coordinator: C.B.). C.B. is supported by an ERC Starting Grant (European Union's Horizon 2020 research and innovation programme, grant agreement n° 679146). A.R. is supported by the Klarman Cell Observatory and the Howard Hughes Medical Institute. J.A.K. is supported by the Austrian Federal Ministry of Education, Science and Research; the Austrian Academy of Sciences; and the European Research Council (European Union's Horizon 2020 research and innovation programme, grant agreements n° 695642 and 693184).

Competing interests

The authors declare the following competing interests:

- Christoph Bock: Co-founder and scientific advisory board member of Aelian Biotechnology; co-inventor on patent applications relating to genome technology.
- Michael Boutros: No competing interests declared
- Laura Clarke: No competing interests declared
- Hans Clevers: Inventor on several patents involving adult stem cell-based organoid technology; chief scientific officer of Hubrecht Organoid Technology (unpaid), co-founder of Surrozen, scientific advisory board member of Kallyope, Merus, and Decibel; nonexecutive board member of Roche and Genentech; scientific advisor of Life Sciences Partners
- Juergen A. Knoblich: Inventor on several patents involving pluripotent stem cell-based organoid technology; co-founder and scientific advisory board member of a:head bio
- Prisca Liberali: No competing interests declared
- Aviv Regev: Co-founder of Celsius Therapeutics; equity holder of Imunitas; scientific advisory board member of Syros Pharmaceuticals, Thermo Fisher Scientific, Asimov, and NeoGene Therapeutics; co-inventor on patent applications filed by the Broad Institute relating to single-cell genomics.
- Anne Rios: No competing interests declared

- Oliver Stegle: No competing interests declared
- Hendrik G Stunnenberg: No competing interests declared
- Sarah A. Teichmann: Consulting for Genentech, Roche; scientific advisory board for Biogen, Foresite Labs, and GlaxoSmithKline
- Robert G.J. Vries: Inventor on a patent involving adult stem cell-based organoid technology. Full-time employee of the Foundation Hubrecht Organoid Technology (HUB), which holds the exclusive license of organoid technology patents

Figures

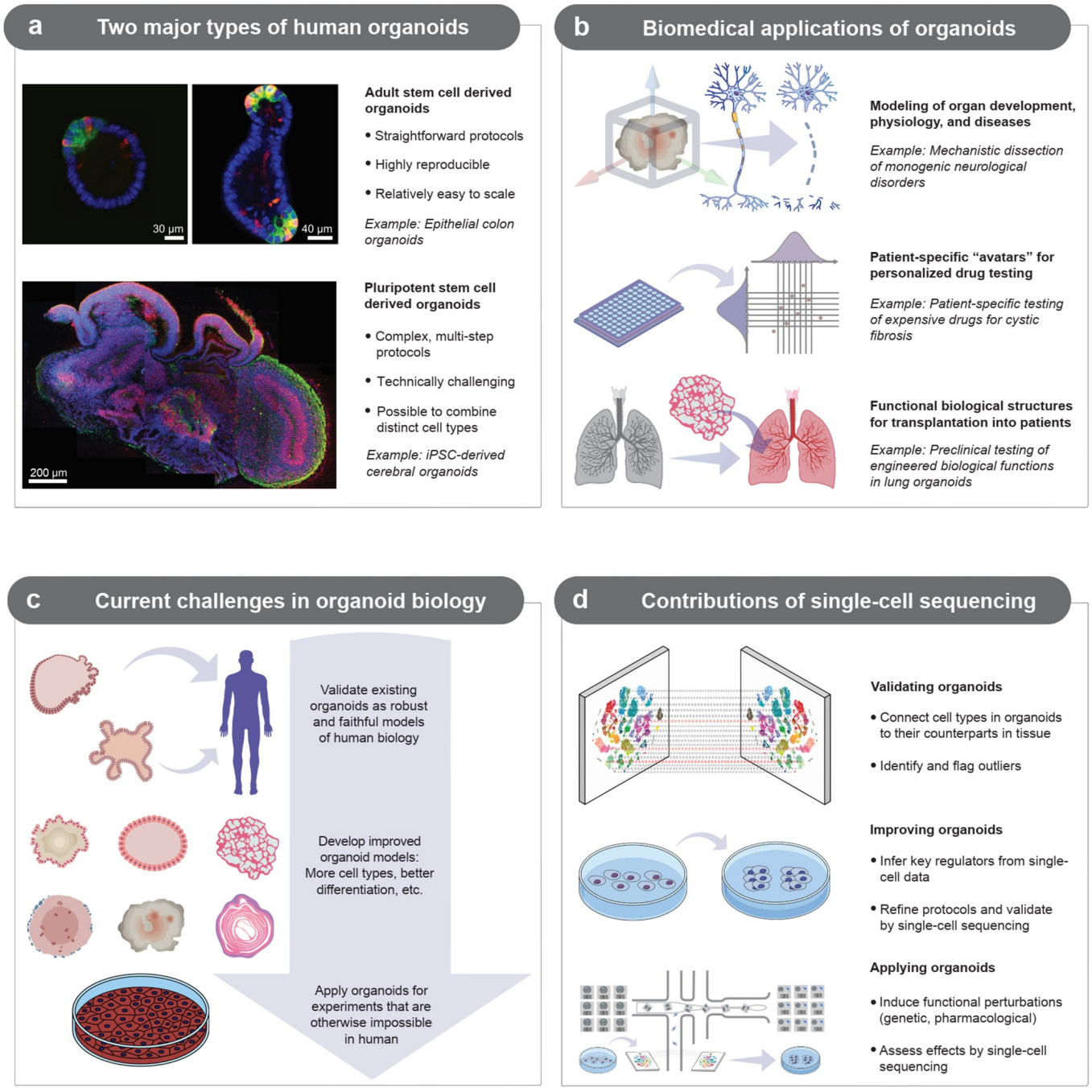

Fig. 1. Combining organoids and single-cell technology for biomedical discovery and regenerative therapy

(a) Organoids can be derived from adult stem cells, which are already committed to a specific cell type, tissue, and/or organ; or they are derived from pluripotent cells, which can give rise to a broad set of cell types but requires more complex differentiation protocols. **(b)** Human organoids are widely useful for *in vitro* modeling of biological functions, drug testing, and regenerative medicine. **(c)** Key challenges for advancing organoid research include characterization/validation of organoids, development of new organoid protocols, and applications of organoids for basic biology and biomedical applications. **(d)** Single-cell technology promises to advance organoid research by enabling systematic validation, by informing organoid protocol development, and by providing a high-resolution readout for functional perturbation experiments in human organoids.

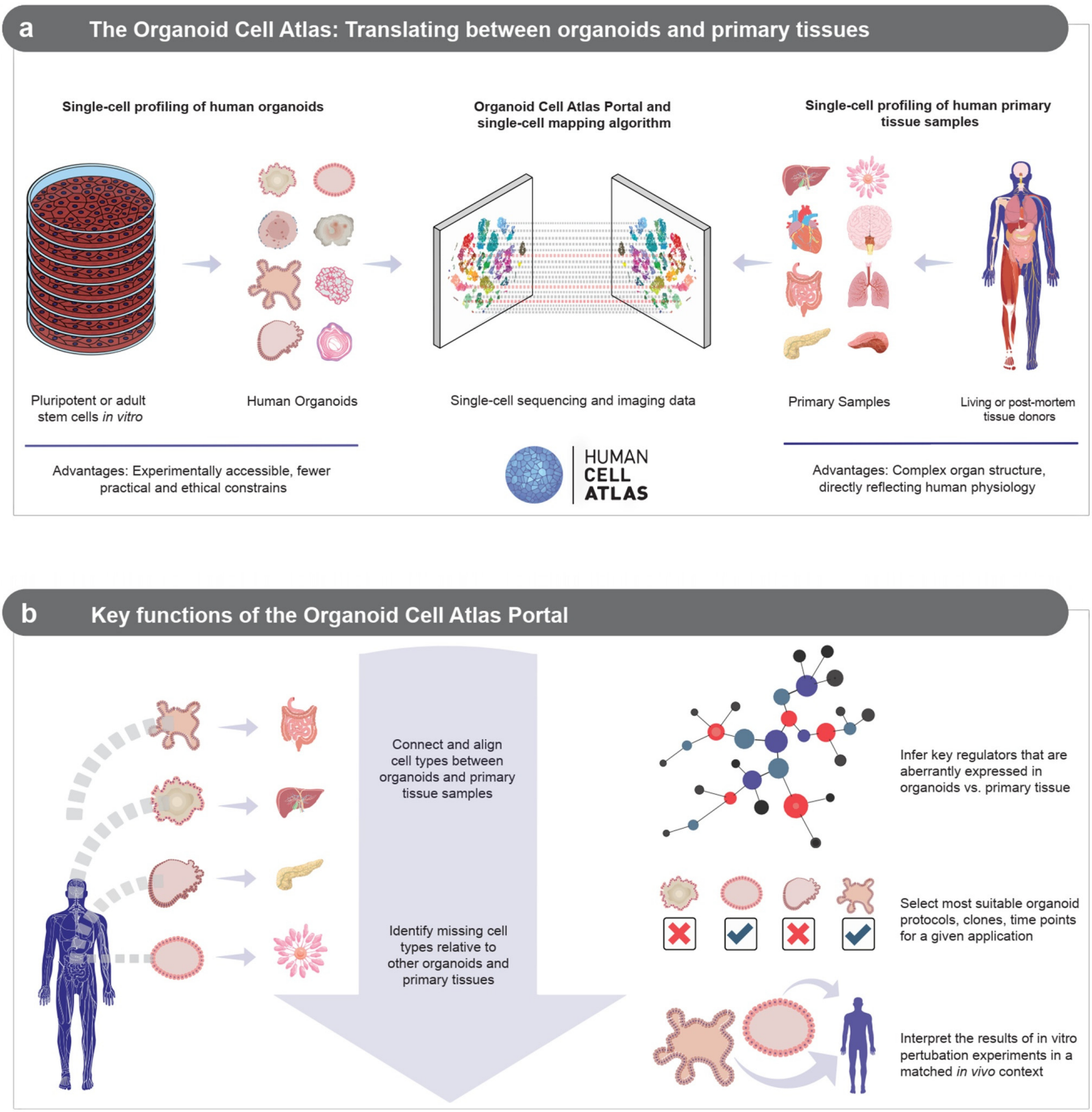

Fig. 2. Connecting single cells in human organoids and in primary tissue samples via the Organoid Cell Atlas

(a) Single-cell profiling of human organoids (left) and of human primary tissue samples (right) provide complementary information. Data integration between single-cell profiles from organoids and primary tissues makes it possible to investigate the same cell type in both models, allowing each approach to play out its strengths. **(b)** The Organoid Cell Atlas Portal will implement key features for analyzing and interpreting single-cell data of human organoids in a biological context provided by HCA profiles of their *in vivo* counterparts.

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