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A roadmap for the Human Developmental Cell Atlas

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Abstract

The Human Developmental Cell Atlas (HDCA) initiative, part of the Human Cell Atlas, aims to create a comprehensive reference map of cells during development. This will be critical to understand normal organogenesis, the impact of mutations, environmental factors and infectious agents on human development, its relevance to congenital and childhood disorders, and the cellular basis of ageing, cancer and regenerative medicine. In this perspective, we outline the HDCA initiative and the challenges of mapping and modelling human development using state-of-the-art technologies in order to create a reference atlas across gestation. Like the Human Genome Project, the HDCA project will integrate the output from a growing community of scientists mapping human development into a unified atlas. We describe the early milestones achieved and the use of human stem cell-derived cultures, organoids and animal models to inform the HDCA, especially for prenatal tissues that are hard to acquire. Finally, we provide a roadmap towards a complete atlas of human development.

Introduction

Historically, most modern developmental biology research has focused on model organisms. Due to practical challenges, human development, from a fertilised ovum to a fully formed fetus at birth, has remained a poorly understood ‘black box’. The implications of a Human Developmental Cell Atlas (HDCA) for understanding human development are far-reaching, as many congenital disorders and childhood cancers may originate during susceptible windows of development¹⁻³. The clinical relevance extends into adulthood for ageing, cancer and applications in regenerative medicine and stem cell therapies⁴⁻⁶. Furthermore, embryonic and fetal stem cells^{7,8} and developmental trajectories provide an essential reference and guide for engineering human stem cell-derived models⁹⁻¹³, organoids¹⁴ and cellular therapies.

Human development begins with a fertilised oocyte that divides and differentiates through pre-implantation, embryonic and fetal stages (**Figure 1**). Early studies began through morphometric and qualitative assessments of human embryos, leading to development of the Carnegie staging

system (**Figure 1**)¹⁵. Advances in imaging, cytometry, and genomics technologies have revealed further insights into the complex spatio-temporal changes during organogenesis¹⁶. Recent progress in single cell profiling technologies has revolutionised our ability to study human development at unprecedented resolution¹⁷. Leveraging these advances to build a comprehensive atlas of human development (from fertilised oocyte to birth) at cellular resolution is an ambitious endeavour similar to the scale of the Human Genome Project (HGP), which required multidisciplinary scientific expertise from disparate fields working together collaboratively. Such a community has arisen from a grassroots assembly of researchers worldwide working as part of the Human Cell Atlas (HCA¹⁸) initiative. Like the HGP, the HCA will be a foundational scientific resource, composed of diverse data types and available freely through browsable and searchable web portals that visualise cells across anatomical space and developmental time.

The HDCA, a strategic focus of HCA¹⁹, is pursued by scientists from individual labs and large national and international research consortia, and is open to all who adhere to its mission and open science values²⁰. The HDCA aims for equity, inclusivity and diversity both in terms of scientific participation and human tissue sample representation. We encourage any interested researcher to become a member, participate, register their study and contribute their data and publication to the HDCA and the HCA²¹.

Building a developmental cell atlas

Successful construction of a HDCA poses enormous scientific challenges, in terms of experimental measurement technologies, computational analysis and visualization algorithms (**Figure 2**). In particular, the dynamic nature of gestation creates challenges for designing a sampling strategy, especially to capture transient morphological changes in the first eight weeks. A major endeavour for the HDCA will be to develop the conceptual and computational framework to capture development with respect to cellular and morphological changes. The HDCA through coordination with the HCA Organoid Network²² will incorporate data from *in vitro* culture model and organoid systems²³ to cautiously infer development between 7 days to 4 post-conception weeks (PCW) when samples are difficult to obtain (**Figure 1b-c**).

The successful delivery of a HDCA will leverage the HGP-initiated restructuring of how large science projects are funded, conducted, coordinated and shared (based on the Fort Lauderdale Principles²⁴) that form the basis for HCA, its committees (e.g. computation, ethics) and ‘Biological Networks’²⁰. This organisational framework has enabled researchers to form large-scale coordinated collaborations across technologies and biological disciplines: developmental biology, embryology, genetics and model systems, computational biology, clinical specialties including *in vitro* fertilization, clinical genetics and pathology, as well as coordination with funders. Partnerships with allied biological networks, including organoid and paediatric atlas projects will facilitate clinical applications (**Supplementary Table 1**).

Ethics, resources and data sharing

Accessing human developmental samples is constrained by general and geographically specific ethico-legal challenges. These include issues relating to donation, access, and research use of legally-defined developing human tissue material, regulatory approvals processes and cultural sensitivities. Research on human embryos and fetuses is supported within European and national regulations, such as the UK National Research Ethics Service (NRES) and the French Agence de Biomédecine. In the UK, studies on preimplantation human embryos up to 14 days are governed by the Human Fertilisation & Embryology Authority (HFEA) and a research ethics committee (e.g., NRES). However, in the United States, research on donated human embryonic and fetal materials has been increasingly restricted over the last two decades, despite the existence of similar regulatory oversight.

Nonetheless, resources to support research in human development such as the UK’s Human Developmental Biology Resource (HDBR²⁵) provide material to researchers. Non-UK recipients of tissue require their own project-specific ethics approval, prior to receipt of material. HDBR provides embryonic and fetal samples from 4-20 PCW with karyotype information and, increasingly, with anonymised maternal DNA and clinical history. Material from fetuses with prenatally diagnosed disorders is also available. The French Human Developmental Cell Atlas (HuDeCA: <https://hudeca.genouest.org>) was recently established and aspires to constitute a comprehensive European resource of human embryonic or early fetal samples.

International sharing of genomic sequencing and clinical data derived from prenatal or paediatric tissue samples is subject to governing data protection regulation that considers live/deceased status, consent regarding research data use and confidentiality. Data from living donors is shared under appropriate access controls. The HCA Ethics Working Group is developing tools, guidance notes (available at²⁶), consent form templates and sampling information for embryonic, fetal and paediatric tissue material, and international data sharing guidance for HDCA.

Mapping development across space and time

Development is intricately orchestrated in three spatial dimensions and gestation time. Human embryogenesis cannot be easily assessed at high resolution *in vivo*²⁷. Time-lapse studies are limited to *in vitro* pre-implantation embryos. The application of high-throughput genomics technologies to dissociated cells and tissue sections *in situ* is beginning to provide data of unprecedented resolution (**Figure 3 and Figure 4**).

Cellular and molecular heterogeneity

Single cell molecular profiles based on RNA, chromatin accessibility, methylation or select protein signatures, have enabled a more nuanced definition of cell types and states. The data underpinning such definitions are increasingly derived from single cell RNA-sequencing (scRNA-seq), barcoded antibodies and accessible chromatin sequencing of dissociated cells^{28,29-28}. Resolving cell types and trajectories at high granularity is aided by full-length scRNA-seq but primarily performed by profiling large numbers of cells. Cell type definition is currently guided by existing knowledge from model organisms and adult cellular profiles, which may not faithfully reflect prenatal cell types, transient cell types only present during development and transitional states of differentiation.

To overcome these challenges, many time points need to be profiled, and defined cell states need to be mapped back into their 3D space over time and functionally characterised. High levels of multiplexing can attain this level of granularity at an affordable cost for a complete HDCA^{30,31}. Molecular profiles, morphology, functional assessment and other features can reflect a cell's multi-faceted state. For example, the transcriptome reflects the present and potential future of a cell,

protein expression captures the immediate past and present state of a cell, chromatin profiles reveal its invariant type and potential for future differentiation, and ontogeny reveals its history.

The field of developmental biology has traditionally drawn on ontogenic relationships to define cell types, but this is challenging in humans where information is captured as snapshots across gestation. CRISPR scarring is only applicable in stem cells, organoid systems and short-term explants^{32,33}. Somatic mutation tracking is the only available technology to definitively determine ontogeny, but is limited by its current lack of scalability^{34,35}. Recent methods that rely on simultaneous measurement of mitochondrial DNA/RNA, transcriptome and open chromatin may overcome this challenge^{36,37}. We anticipate the field moving towards a consensus cell ontology that integrates multi-modal single-cell profiling data as well as legacy knowledge of embryonic cell type definitions augmented by information from diverse animal models.

Mapping cells in 2D and 3D

Spatial genomics methods to measure RNA in tissue sections typically offer a trade-off: high resolution (single cell and subcellular) methods that typically measure hundreds of transcripts or whole transcriptome profiles at multi-cellular level^{38,39}. This trade-off can be mitigated by integration with single-cell profiles from dissociated cells, expanding the genomic coverage by predicting spatial expression of unmeasured genes, or enhancing resolution by deconvolution of multi-cellular measurements. Tissue clearing methods to render organs transparent⁴⁰ combined with whole-mount protein immunostaining and RNA single-molecule FISH^{41,42} can now provide 3D molecular profiling at cellular or subcellular resolution using light-sheet microscopy⁴³⁻⁴⁵. Increasing multiplex capacity and use of artificial intelligence/machine learning algorithms to overcome data analytical challenges was successfully deployed to image whole-organismal vasculature following tissue clearing^{46,47}.

Biophysical methods and live imaging

Mounting evidence from *Drosophila* and other models shows that mechanical forces play a key role in development processes and tissue morphogenesis⁴⁸. Surface tension and pressure can be measured in single cells of preimplantation mouse embryos⁴⁹. Adapting these technologies to

human pre-implantation embryos and stem cell-based embryo models⁵⁰ can build a spatiotemporal mechanical atlas.

Positional landmarks in development

A standard coordinate system for locations in the human body (a common coordinate framework; CCF) is crucial for the HCA and HDCA⁵¹. Two types of systems are useful: absolute, similar to postcode/zip-code addresses, and relative, similar to a landmark-based address system. CCF anatomical ‘postcodes’ enable integration of multi-modal datasets of different spatial and longitudinal resolution. The Allen Mouse Brain Reference Atlas v3 provides a CCF of 3D anatomical features and local features grouped in a hierarchy to facilitate multilevel analysis of the mouse brain. Efforts are currently underway to establish CCFs for adult human organs within the NIH-HuBMAP initiative. The HDCA will need to develop a CCF that incorporates space and time, as well as cell movement and patterns during organogenesis based on existing macro-level 3D coordinates for human embryos, such as the HDBR Atlas (<http://hdbratlas.org/>) and the Transparent Human Embryo (<https://transparent-human-embryo.com/>).

Computation and data visualisation

Among the key algorithmic challenges to integrating data into a developmental atlas are i) mapping cells with more intermediate states compared to adult counterparts; ii) inferring time orderings and lineage relations, including branching lineages and multiple paths converging on the same outcome; iii) inferring spatial movement of cells; iv) building a temporal series of CCF, each as a probabilistic model for a time window as well as a model for their morphing along space and time⁵²; v) mapping across modalities and time points (e.g. chromatin states in one time window to RNA and protein levels of another), and vi) regulatory and molecular network inference within and across cells. New theories and insights from multiple fields will be required to model the mechanisms underpinning tissue formation and growth. It is likely that additional emergent properties of cells and their ecosystems will be discovered using interdisciplinary approaches. These will need new vocabularies, ontologies and modelling approaches to be understood. The HDCA community must also apply FAIR principles to help ensure reproducibility and data accessibility⁵³.

Computational integration of multi-omics data for ‘Google maps’-like visualisation, such as the Open Microscopy Environment (<https://www.openmicroscopy.org/>) will enable zooming to the single cell level from a large-volume tissue view. Additional complexity will combine visualisations from imaging and sequencing data. Sophisticated abstraction of raw data and integration across modalities, anchored by a developmental CCF will be essential. Links to clinical relevance and applications will enhance the utility of the atlas.

Emerging cell atlases of human development

The advantages of whole tissue/organ profiling compared to lineage-centric analysis include comprehensive cellular analysis and the discovery of emergent biological properties. For example, the developing liver functions as a haematopoietic organ during early gestation until mid-second trimester, before it functionally transitions into a metabolic organ like the adult liver⁵⁴. To meet the high demand for erythropoiesis during development, the first trimester human skin and adrenal glands can also support erythrocyte maturation^{54,55}.

In stark contrast to our terrestrial postnatal life, the human embryo/fetus exists in an aquatic environment. Our lung, gut and skin are exposed to amniotic fluid. In contrast to postnatal lung, the developing lung does not perform oxygen transfer or receive the same volume of blood through the pulmonary veins. The impact of these physiological factors on individual tissues and the role of placenta and maternal decidua in supporting human embryogenesis and fetal life are emerging^{56,57}.

Organ atlases of brain, gut, heart, liver, kidney, placenta, thymus and skin (**Figure 4**) underscore the importance of studying human samples and reveal the unique aspects of human development not conserved with animal model systems^{58–61}. These include timelines of development during gestation, cell type markers and expression pattern of transcription factors between mouse and human organs^{62,63}.

The specification of functional tissue niches occurs during both prenatal and postnatal life. Fetal gut studies highlight the importance of interactions between the epithelial and mesenchymal compartments to allow the formation of villi and have identified fetal gut transcription factors that

are aberrantly activated in paediatric Crohn's disease⁶⁴. Comparison between developing and adult kidney demonstrated the establishment of a dedicated spatial zonation pattern that protects against uropathogenic bacterial challenges postnatally^{61,65}. Single-cell transcriptomics of germ cells during development have revealed important insights into the main pathways controlling their differentiation^{66,67} with ongoing studies focused on unravelling the regulatory mechanisms of sex determination (<https://hugodeca-project.eu>).

Early developmental studies of the brain have focused on human and primate cortical development⁶⁸⁻⁷⁰. The developing human and rodent midbrain, which contains the clinically relevant dopaminergic cell groups that are lost in Parkinson's disease, has also been extensively studied^{63,71,72}, as has the developing mouse spinal cord and cerebellum^{73,74}, the hypothalamic arcuate nucleus and the diencephalon⁷⁵.

Atlases of distributed systems such as the immune system have been initiated, detailing haematopoietic organs such as the yolk sac^{76,77} and liver⁵⁴, lymphoid tissues such as thymus where T cells differentiate⁷⁸ and non-lymphoid tissues such as skin and kidney where immune cells reside. These studies revealed an intrinsic change in the differentiation potential of haematopoietic stem progenitor cells with gestational time, together with the importance of the local tissue microenvironment for blood and immune cell development.

Model organisms and culture systems

Our understanding of human development has been largely inferred from studies on animal model systems that are not always conserved across species (**Figure 1**)⁷⁹. Two recent studies contrast the kinetics of development between human and mouse, highlighting the need for caution in interpreting heterospecific graft studies and findings from non-primate preclinical models^{80,81}. However, the feasibility of perturbation and in-depth mechanistic studies using animal models and culture systems provide a valuable scaffold and complement the HDCA, particularly for the immediate weeks after implantation where human samples are inaccessible.

Single cell molecular profiling has transformed many aspects of developmental biology research across all major model organisms⁸²⁻⁸⁶ providing new mechanistic insights into fundamental

biological processes including the early specification of germ layers and diversification of early cardiovascular cells^{29,87}. Comparative biology has the potential to make major contributions to cell ontology. The availability of parallel human and model species data will support expanded cross-species analyses. Computational analysis can align cells and inferred lineages across species to extrapolate findings from non-primate models and help optimise animal models of normal and pathological human development. From a computational perspective, it will be important to develop tools for better annotation of 3' and 5' UTRs of animal model data as most scRNA-sequencing technologies capture only these regions. Development of computational tools that can robustly map developmental trajectories across species that can account for different developmental kinetics between cell types within and between species will be required. Comparative studies of human and mouse pre-implantation and gastrulation embryos indeed revealed conserved and divergent transcriptional programs. For example, *Klf2* expression in mouse embryo-fated epiblast progenitor cells is not observed in humans; and by contrast, *KLF17* is enriched in human but not mouse epiblast⁸⁸.

Self-organization of human embryonic tissue can be captured from the earliest moments *in vitro*^{50,89}, and extended to gastrulation, anterior-posterior embryonic patterning, and early phases of somitogenesis¹¹. The recent human gastrulation embryo dataset will be informative as a benchmark to further refine *in vitro* directed differentiation of human cells, including gastruloid models¹¹. Other processes during organogenesis can also be monitored, including clock control of somite segmentation^{90,91}, boundary formations during hepato-biliary-pancreatic organ budding⁹² and patterning of the neural tube. Protocols are now established to mimic development of diverse human tissues that exhibit morphologies and physiologic functionalities of developing human tissues. Such organoid systems include hair-bearing skin⁹³; small intestine with a crypt-villus axis⁹⁴; region-specific⁹⁵ and multi-region⁹⁶ brain tissue modelling neurogenesis, neural migration, and synapse formation; multi-layered neural retina with photoreception responses⁹⁷; and arterio-venous specification during blood vessel development⁹⁸.

A comprehensive reference atlas of cell types and states present during human development will be critical to benchmark stem cell-derived organoids. Such roadmap comparisons will highlight similarities⁶⁹, deficiencies⁹⁹, and define strategies for improving organoids for disease modelling.

In the future, high-fidelity human stem cell-derived human organoids and single-cell multi-omic modalities will be powerful tools to understand mechanisms controlling human organogenesis.

Clinical relevance and applications

The interaction of genotype and environment leading to phenotype underlies developmental disorders. A range of childhood and adult disorders have their origins in prenatal life (**Figure 5**). These include structural birth defects¹⁰⁰, neurodevelopmental disorders including schizophrenia¹⁰¹, childhood cancers^{2,65}, inborn errors of immunity¹⁰², infertility and differences of sex development¹⁰³, as well as many paediatric disorders¹⁰⁴. Thousands of rare genetic diseases can each present a spectrum of perturbed developmental sequelae at birth, sometimes differing widely in medical presentation even when classified as the same disease¹⁰⁵. As examples, Down syndrome (trisomy 21)¹⁰⁶ and 22q11.2 deletion syndrome¹⁰⁷ separately present significant risks for schizophrenia, Alzheimer's disease, and hypothyroidism starting in adolescence¹⁰⁸. Identifying the aetiology of developmental disorders and the effects of maternal genotype, paternal age and other external risk factors such as diet, alcohol, toxins, endocrine disruptors and pathogens have been hampered by our limited understanding of normal human development.

Development atlases are also unravelling the pathogenesis of childhood cancers (**Figure 5**). Paediatric and adult brain tumours in their early stages often present impaired developmental programs within tumour cells^{109,110}. Comparing the expression profile of tumour cells with HDCA can identify the cancer cell of origin and its oncogenic pathways. For example, a single-cell atlas of the developing mouse cerebellum was used to dissect subtypes of human medulloblastoma, a paediatric brain tumour^{2,111} and cell states during nephrogenesis discerned the developmental cellular origin of Wilms tumour⁶⁵. High resolution mapping of developing immune cells will inform the molecular and extent of disease phenotypes of childhood leukaemias and primary immunodeficiencies.

Many adult cancers also recapitulate a dysregulated version of human developmental programs¹¹². The acquisition of early developmental molecular programmes is characteristic of malignant pathology and a previously unrecognised hallmark of immunological disease and cancer immune environment^{113,114}. HDCA data have also facilitated our understanding of differential susceptibility

of adult and prenatal cells to SARS-CoV2 through examination of viral entry receptor and protease expression in a wide range of organs¹¹⁵.

Cell and tissue engineering for clinical therapies and regenerative medicine are areas with enormous potential for the direct utility of the HDCA. Cell therapies derived from human pluripotent stem cells are now entering early clinical trials for Parkinson's disease¹¹⁶ using protocols that were refined based on developmental studies of midbrain dopaminergic neurons⁷². Similar approaches are being followed to develop a range of other stem cell products for human trials¹¹⁷. Haematopoietic stem cell (HSC) transplantation is an established and widely used treatment for many haematological and increasingly non-haematological disorders. Leveraging the potency factors of fetal HSCs could have significant benefit to patients receiving HSC transplants.

Towards a whole embryo atlas

The initial HCA White paper emphasised 12 distinct organ systems within the human body and highlighted the importance of a developmental cell atlas. Integrated multi-organ analyses will provide novel insights into tissue microenvironment shaping resident epithelial, stroma and immune cells and the cellular heterogeneity of innervating blood vessels, lymphatics and peripheral nerves. Eventually, this may illuminate system-level lineage development and cell fate decision across an entire organism. The datasets from human developmental organ-based profiling were critical in interpreting recent multi-organ developmental atlases^{55,118}.

There are several large-scale organ-based studies by HDCA researchers. These include NIH BRAIN Initiative BICCN consortium focusing on the developing human cortex, the Swedish HCA consortium performing large-scale scRNA-seq, ATAC-seq and spatial-omic analysis of the developing human brain, heart¹¹⁹ and lung during the first trimester, the French HuDeCA consortium to map eight first trimester human organs using 3D-imaging and scRNA-seq, the EU H2020-funded developing brain (Braintime) and gonad (HUGODECA), the NIH Developmental Genotype-Tissue Expression (dGTEX¹²⁰) and Wellcome and MRC-funded consortia in the UK. The logical next step will be to coordinate these efforts and extend the current approach to contextualise the development of different cell lineages across all organs.

However, multi-organ approaches do not permit the analysis of distributed tissue networks as a continuum from a single donor sample. Whole embryo analysis has been limited to very early pre-implantation samples^{88,121,122} and one gastrulation stage embryo¹²³. Multi-omics suspension and spatial-genomics profiling of anatomically dissected units from whole human embryos at 6/7 PCW is being undertaken by the UK HDCA researchers. We anticipate a first whole human embryo profiling within the next two years. Based on existing HDCA data and the rapid changes during early development, we propose a minimum of three replicates for each biologically relevant gestation period (e.g. each week from 6 PCW). All such data produced and shared by the global research community, formally registered with the HCA or not, contributes to the HDCA. Defining a universal organising framework for this data will enable it to be unified into a complete atlas that will be a transformative resource for the research and clinical communities.

Figure and table legends

Figure 1: Human embryo development and model systems

- a. Timeline of human development from fertilization to birth.
- b. *In vitro* model systems to study early embryonic development.
- c. Experimental model systems to study development, including *D. melanogaster*, *D. rerio*, *X. laevis*, *G. gallus*, *M. musculus*, cell culture and organoids, and their amenability to facilitate various aspects of scientific study.

Figure 2: The Human Developmental Cell Atlas: how to build it and what will it provide?

- a. 'How to build an atlas' modules, including an interdisciplinary team, multi-modal technologies, and integration of data across platforms.
- b. Key features of the Human Development Cell Atlas. Single cell measurements across three-dimensional space, alongside a fourth dimension of time, allow for capture of dynamic developmental processes including cell proliferation, migration and regulation.

- c. Utility and applications of the Human Development Cell Atlas: cellular and molecular biological insights applied to advance regenerative medicine, tissue engineering and therapeutics.

Figure 3: Multi-omics profiling and data integration

- a. Organ or anatomical unit profiling of a prenatal embryo derived from multiple germ layers.
- b. Single cell atlas technologies by relative resolution and genome scale.
- c. Integration of datasets from different technologies (e.g., spatial transcriptomics, single-cell RNA sequencing, targeted *in situ* sequencing) to profile organs or whole embryos.

Figure 4: Publications registered with the Human Development Cell Atlas. There are 48 researchers from 13 countries currently registered with the HDCA. Developmental datasets are contributed to public repositories including the HCA Data Coordination Portal.

Figure 5: Clinical relevance and applications of the Human Developmental Cell Atlas

- a. A timeline of brain development across human life, with examples of diseases with onset at different gestational stages and ages.
- b. How a single cell atlas with temporal and spatial information can be used as a reference to understand disease state.

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Author contributions

M.H.; S.T. and A.R. conceived the idea, co-ordinated the writing process, wrote parts of the paper and edited all sections. A.H. designed and created the figures. All other authors wrote parts of the paper and provided feedback on all parts.

Conflict of interest

A.R. is a co-founder and equity holder of Celsius Therapeutics, an equity holder in Immunitas, and was an SAB member of ThermoFisher Scientific, Syros Pharmaceuticals, Neogene Therapeutics and Asimov until July 31, 2020. From August 1, 2020, A.R. and O.R-R. are employees of Genentech. S.A.T. has consulted for Genentech and Roche, and is a remunerated member of Scientific Advisory Boards for GlaxoSmithKline, Biogen and Foresite Labs. J.L. is a scientific advisor for 10x Genomics. All other authors declare no competing interests.

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Descriptions of the 5-10% most important references

Asp, M et al. A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the Developing Human Heart. *Cell*. vol. 179(7):1647-1660.e (2019).

- **A temporal and three-dimensional spatial map of the developing human heart from the first trimester by using a combination of transcriptome wide single cell RNA sequencing and spatial transcriptomics methods with cellular validation by in situ sequencing.**

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- **A three-dimensional map of first trimester human development by tissue clearing and lightsheet imaging, providing high resolution images of the developing cardiopulmonary, vascular, peripheral nervous, muscular and urogenital systems, unveiling insights into complex processes such as skin innervation and differential vascularisation of male and female genital systems.**

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- **This review highlights the potential utility of single-cell genomics to optimise cell and tissue engineering, with a focus on emerging methodologies that can guide this process, such as transcription factor combinatorics, spatial reconstruction, CRISPR-Cas9 screens and lineage-coupled transcriptomics.**

Cao, J. et al. A human cell atlas of fetal gene expression. *Science* 370, (2020).

- **A set of two studies on integrating single cell gene expression (this study) and chromatin accessibility (Domcke, S. et al. 2020) from 15 first and second trimester human organs.**

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- **Comparative single cell analyses of fetal kidneys, paediatric, adult kidneys and Wilm’s tumours, demonstrating the origin of Wilm’s tumour as aberrant nephron development.**

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Figure 1

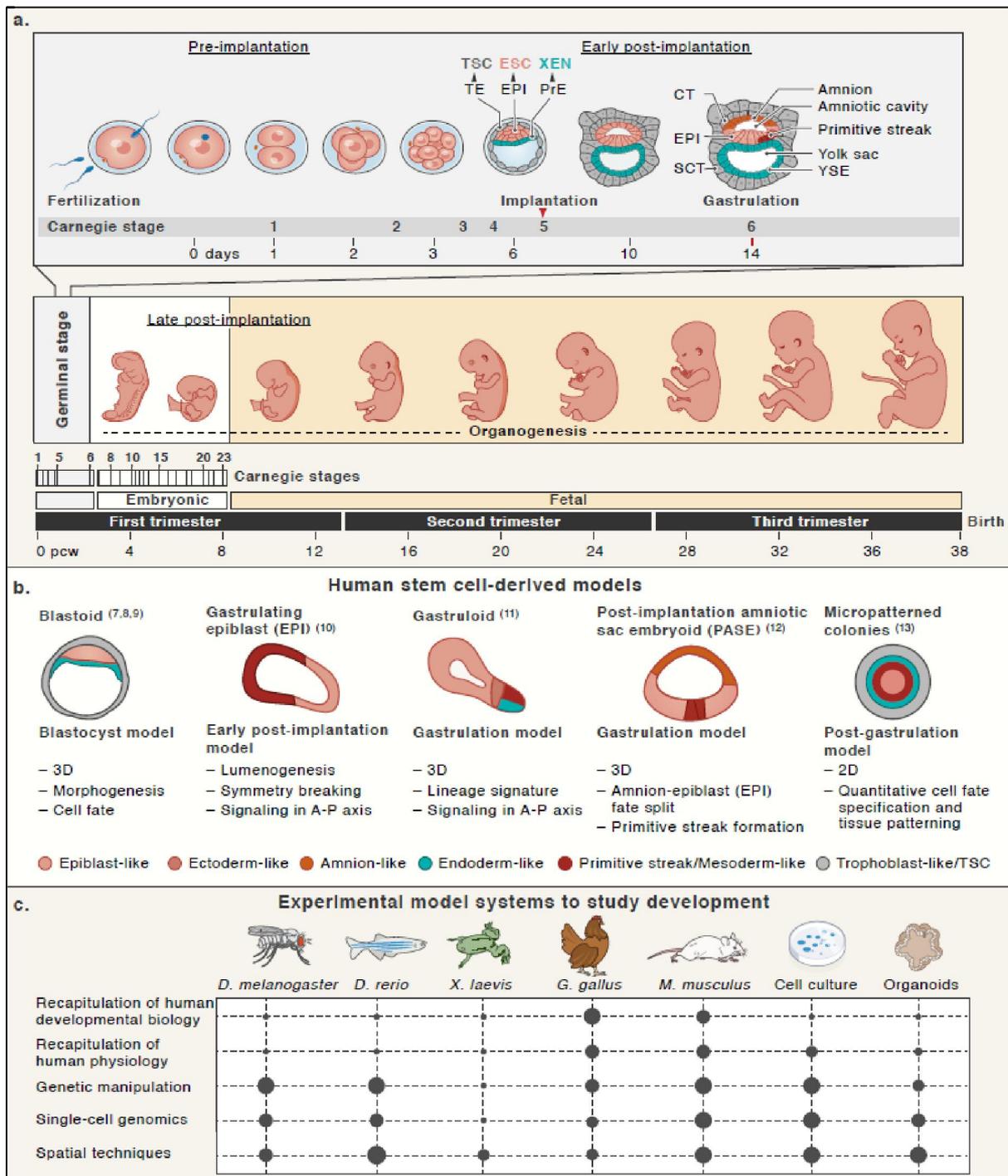


Figure 2

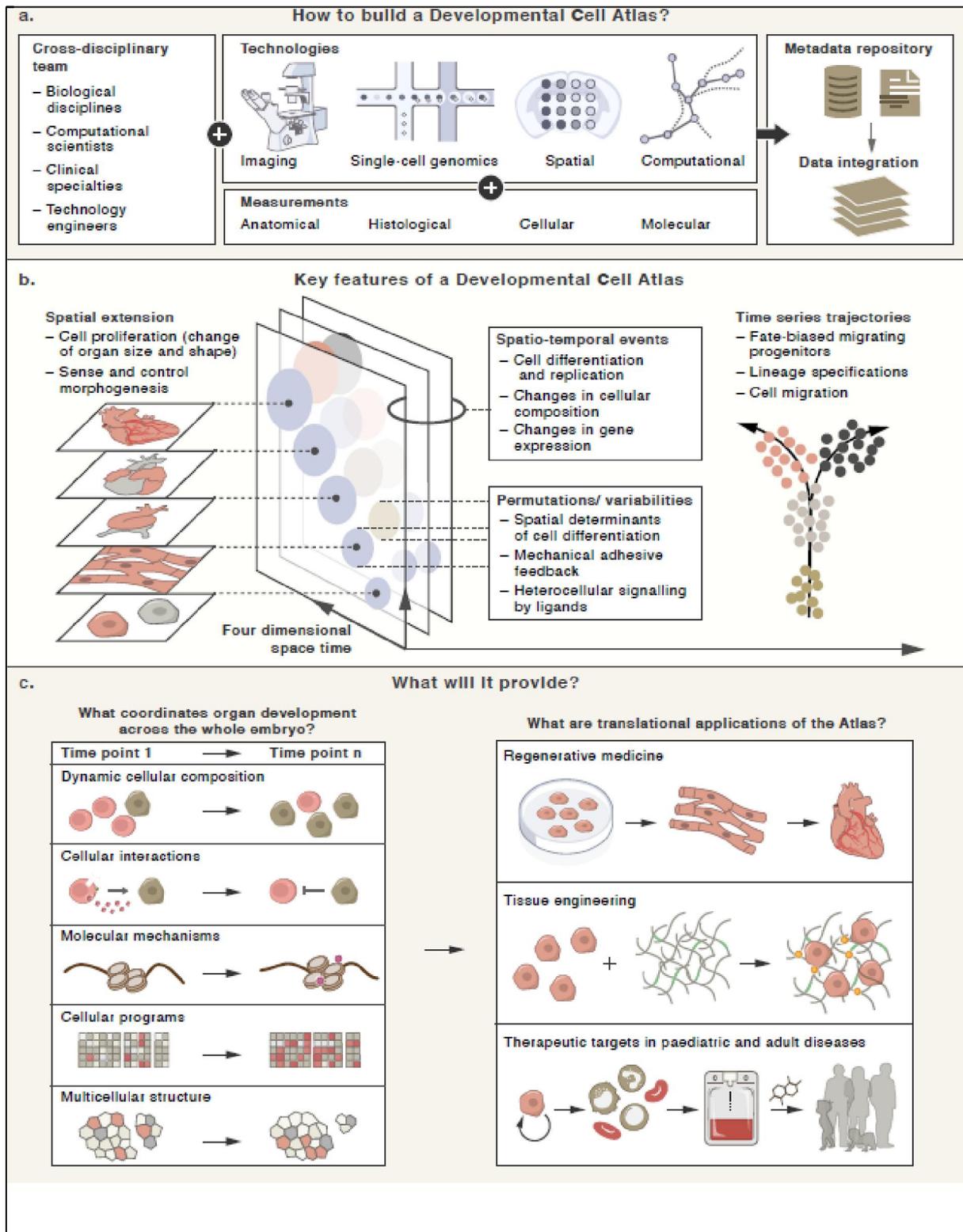


Figure 3

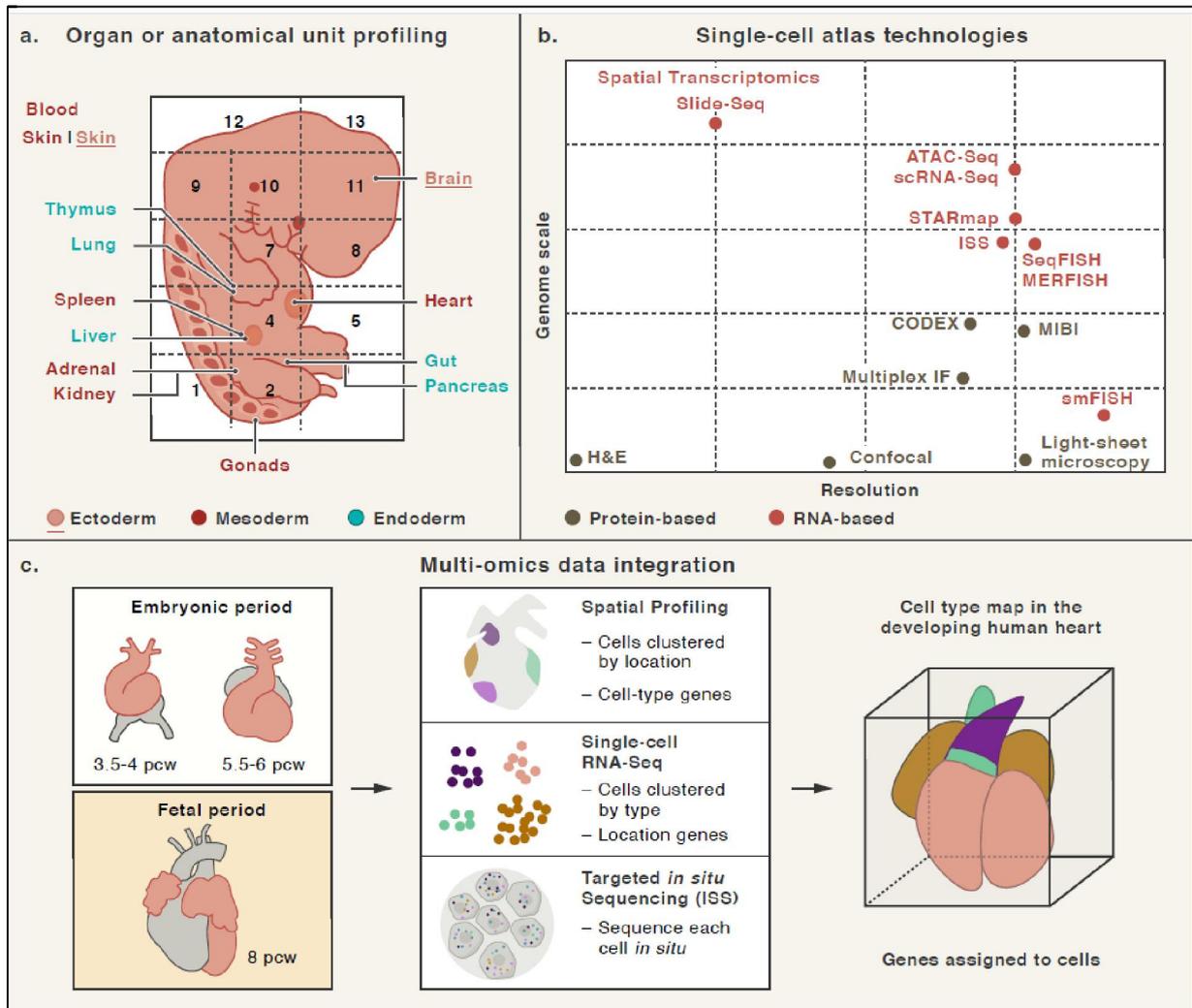


Figure 4

Organ	Main highlights	Publications
Brain 	<ul style="list-style-type: none"> – First and second-trimester – Specific brain regions studied, including prefrontal cortex and neocortex – Developmental trajectories of cells are traced – Mechanisms underlying neurons generation and circuit formation are characterised 	Pollen et al., 2014 ⁽⁵⁹⁾ Han et al., 2020 ⁽⁶⁰⁾ La Manno et al., 2016 ⁽⁶³⁾
Gut 	<ul style="list-style-type: none"> – First and second-trimester, organoids – Transcriptomes of cycling epithelial precursor cells are profiled – Evaluation of the impact of mesenchymal cells on LGR5 stem cells – Comparison of transcriptomes of ex vivo tissues and in vitro fetal organoids – Comparison of transcriptome profiles from paediatric Crohn's disease epithelium with matched healthy controls 	Czenwinski et al., 2020 ⁽⁵⁸⁾ Han et al., 2020 ⁽⁶⁰⁾ Elmentaite et al., 2020 ⁽⁶⁴⁾
Heart 	<ul style="list-style-type: none"> – First and second-trimester – Identification of unique gene profiles that correspond to distinct anatomical regions in each developmental stage – Integration of scRNA-Seq and spatial data – Generation of a web resource of the human developing heart 	Suryawanshi et al., 2020 ⁽⁵⁷⁾ Han et al., 2020 ⁽⁶⁰⁾ Cui et al., 2019 ⁽⁶²⁾ Asp et al., 2019 ⁽¹¹⁹⁾
Liver/ Fetal haematopoiesis 	<ul style="list-style-type: none"> – First and second-trimester – Identification of the repertoire of human blood and immune cells – Identification of differentiation trajectories from HSC/MPPs – Evaluation of the impact of tissue microenvironment on blood and immune cell development 	Popescu et al., 2019 ⁽⁵⁴⁾ Han et al., 2020 ⁽⁶⁰⁾
Kidney 	<ul style="list-style-type: none"> – First-trimester – Identification of both known and unknown transcription factors associated with nephron development – Characterisation of myeloid and lymphoid populations present during fetal development 	Popescu et al., 2019 ⁽⁵⁴⁾ Han et al., 2020 ⁽⁶⁰⁾ Stewart et al., 2019 ⁽⁶¹⁾ Young et al., 2018 ⁽⁶⁵⁾
Placenta 	<ul style="list-style-type: none"> – First-trimester – Cellular organisation of the decidua and placenta is characterised – Identification of perivascular and stromal cellular subsets – Development of a repository of ligand-receptor complexes – Development of a statistical tool to predict the cell-type specificity of cell-cell communication via receptor-ligand interactions 	Vento-Tormo et al., 2018 ⁽⁵⁶⁾
Thymus 	<ul style="list-style-type: none"> – First and second-trimester, paediatric – Identification of more than 50 different cell states – Identification of novel subpopulations of thymic fibroblasts and epithelial cells – Identification of the cellular network of the thymic niche for T cell development 	Park et al., 2020 ⁽⁷⁸⁾
Skin 	<ul style="list-style-type: none"> – First-trimester – Identification of physiological erythropoiesis – Enrichment of innate immune cells – Co-optation of developmental programmes identified in adult inflammatory skin diseases 	Popescu et al., 2019 ⁽⁵⁴⁾ Reynolds et al., 2021 ⁽¹¹⁴⁾
Multi-organ 	<ul style="list-style-type: none"> – First and second trimester – Integrated analyses of transcriptomes and chromatin accessibility from multiple fetal organs performed – These include brain, heart, lung, gut, kidney, adrenals, stomach, pancreas, spleen, gonads, muscle, eye and skin 	Han et al., 2020 ⁽⁶⁰⁾ Cao et al., 2020 ⁽⁸⁵⁾ Domcke et al., 2020 ⁽¹¹⁸⁾

<https://www.humancellatlas.org/publications>

Figure 5

