Human Developmental Cell Atlas: milestones achieved and the roadmap ahead

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Abstract

The Human Developmental Cell Atlas (HDCA), as part of the Human Cell Atlas, aims to generate a comprehensive reference map of cells during development. This detailed study of development will be critical for understanding normal organogenesis, the impact of mutations, environmental factors and infectious agents on congenital and childhood disorders, and the molecular cellular basis of ageing, cancer and regenerative medicine. In this perspective, we outline the challenges of mapping and modelling human development using state of the art technologies to create a reference atlas across gestation for scientific and clinical benefit. We discuss the potential value of HDCA to enhance human pluripotent stem cell-derived organoid model systems and, in turn, the use of organoids and animal models to inform HDCA. Finally, we provide a roadmap towards a complete atlas of human development.

Introduction

Historically, most modern developmental biology research focused, by necessity, on model organisms. Due to practical challenges, human development, from a fertilized ovum to a fully formed fetus at birth, has remained a poorly understood 'black box'. The implications for understanding human development are far-reaching, as many congenital disorders and childhood cancers may originate during susceptible windows of development [1–4]. The clinical relevance extends into adulthood for ageing, cancer and applications in regenerative medicine and stem cell therapy [5,6,7]. Furthermore, embryonic and fetal stem cells and developmental trajectories provide an essential reference and guide for engineering pluripotent stem cell (PSC)-derived organoids [8]. For these reasons, a cell atlas of human development will have far-reaching impacts that enhance developmental biology research based on model organisms.

Early studies of human embryogenesis began through morphometric and qualitative assessments of human embryos (**Figure 1**). The Carnegie staging system, a valuable resource that is still widely used, is one example that emerged from these pioneering studies [9]. Advances in imaging, cytometry and genomics technologies revealed further insights into the complex four-dimensional spatio-temporal changes and cellular architecture during organogenesis [10].

There are several basic questions we still do not have answers for: what is the cellular composition of the developing human and how does it change dynamically across tissue (space) and gestation (time)? What are the cellular interactions and molecular mechanisms coordinating organ development across the whole embryo? How does developing a tissue or organ differ from maintaining a fully formed tissue?

93 Recent progress in single cell profiling technologies has revolutionised our ability to study human
94 development at unprecedented resolution [11]. Computational methods for single cell genomics data
95 collected from multiple organs and developmental stages have enabled us to define the wealth of
96 developmental cell states and infer developmental trajectories of transitional populations between
97 them. Although data is collected from serial static snapshots across development, because the
98 process is asynchronous, computational algorithms can infer both continuous temporal
99 progressions and the underlying regulatory programs driving them [12,13]. Emerging spatial profiling
100 methods now allow us to map the temporal progression in 2- and 3D spatial context [14].
101
102 Leveraging these advances to build a comprehensive atlas of human development at cellular
103 resolution is an ambitious endeavour, which requires multidisciplinary scientific expertise from
104 disparate fields working together collaboratively at scale. Such a community has now emerged
105 from a grassroots assembly of researchers worldwide working as part of the Human Cell Atlas
106 (HCA) initiative (https://www.humancellatlas.org). Human Developmental Cell Atlas (HDCA), a
107 strategic focus area of HCA [15], is pursued by scientists from both individual labs and major national
108 and international research consortia, and is open to all who adhere to its mission and values.
109
110 *What is a developmental cell atlas?*
111 Reminiscent of the Greek god Atlas, the developmental cell atlas will hold measurements and
112 information about the cells of the developing human, from the earliest stage through fetal life up
113 to birth, spanning multiple modalities that can be used as reference and for interrogation to derive
114 new understanding. From these measurements, the atlas will abstract the census of cells
115 characteristically present both at each time point and canonical spatial coordinate along

116 development. It will map their temporal relations through the processes of differentiation and
117 migration, their different molecular and physical characteristics (such as RNA, chromatin,
118 metabolite, protein profiles and mechanical properties) organizing into programs that characterize
119 their types and states, and their inter-relations across tissues and time. Given data from diverse
120 individuals, the atlas can also address the extent of variation in development, and some of its
121 genetic underpinnings.
122
123 The developmental cell atlas will exist in both tangible and intangible formats. Projected
124 visualisation of cells during development that can be navigated across anatomical space and
125 development time is intuitive and tangible to the human mind. The vast quantities of complex and
126 rich human development data can be explored, mined and fed to computer algorithms for
127 derivative information in powerful formats that are more intangible to the human mind. The latter
128 is analogous to the potential use of data gathered from social media, the internet, physical and
129 purchasing activities to derive information patterns about people and societies that may not be
130 apparent.
131
132 *How does a developmental cell atlas differ from an organ atlas?*
133 Building a developmental cell atlas is particularly challenging, since in embryos, organs are highly
134 dynamic and both their cellular composition and morphological form changes almost
135 continuously. Cells proliferate and organ size increases, organ shape changes, new cell types
136 emerge and are added during differentiation, while others (such as many progenitors) disappear.
137 Cells also move and migrate extensively within and between morphological structures, for
138 example in the central nervous system [16]. Migration is particularly striking for immune cells which,

139 from their first derivation in the yolk sac, colonize all tissues in the body, as do neural crest cells
140 after segregating from the neural tube in a separate lineage. How can such cells be tracked in space
141 and time, and their lineages reconstructed? How are neuronal, lymphatic and vascular endothelial
142 networks established and then function in an integrated manner? This leads to unique challenges
143 in appropriate foundational concepts, sampling strategies, measurement technologies and
144 computational algorithms.
145
146 At the heart of what makes the developmental atlas unique is its dynamic temporal nature. Prenatal
147 development extends over 9 months in humans and continues for years after birth [8,17,18]. Contrary
148 to more static frameworks emerging for adult organ atlases, every basic entity in the developmental
149 atlas needs to be redefined in a dynamic manner. Cell types need to be defined within the
150 appropriate time frame, connected to their progenitors and progeny. Coordinate frameworks need
151 to be defined spatio-temporally, where each coordinate has both spatial and temporal relations.
152 The challenges posed by the temporal nature of the developmental atlas are also its key strength
153 as dynamics can be powerfully harnessed to elucidate the regulatory mechanisms underlying these
154 processes. Understanding the mechanisms that endow developing cells with their plasticity can be
155 employed to improve regenerative therapies and will provide insight into how cancer cells exploit
156 this plasticity to become malignant. Pinpointing the alleles and regulators underlying congenital
157 disorders can help indicate therapeutic strategies [19].
158
159 *How do we build a developmental cell atlas?*
160 Successful construction of a human developmental cell atlas poses enormous practical challenges,
161 both in terms of experimental measurement technologies and in computational analysis and

visualization algorithms (**Figure 2**). In particular, its dynamic nature creates challenges for designing a sampling strategy especially during early gestation, when dramatic morphological changes can occur over mere hours. Due to the challenges posed, model systems that allow for higher temporal resolution can fill in the gaps where samples are difficult to access (**Figure 1b-c**).

The successful delivery of a reference atlas of human development requires a radical restructure at scale of how science is funded, conducted, coordinated and shared. Collaborations across biological disciplines: developmental biology, embryology, genetics, model systems; clinical specialties: maternal/fetal health, pediatrics, *in vitro* fertilization, clinical genetics, histology; technology, including imaging and genomics; and computational biology, among many others, are essential. Access to tissue resources is a prerequisite, which can be constrained by substantial ethical concerns relating to embryonic and pediatric tissue procurement and handling, as well as legalities that differ across international boundaries.

Ethics, resources and data sharing

Building the HDCA presents a number of general ethico-legal challenges, as well as geographically specific ones. These include issues relating to donation, access, and research use of legally-defined developing human tissue material, regulatory approvals processes and cultural sensitivities. In the United States, the use of donated human fetal tissue for research has again become more restricted, due to additional oversight recently imposed by the US Department of Health and Human Services (HHS). Research on human embryos and fetuses is supported within European and individual nations' regulations such as the UK National Research Ethics Service (NRES) and the French Agence de Biomédecine. In the UK, studies on preimplantation human

185 embryos up to 14 days are governed by licensing from the government regulatory body, the Human
186 Fertilisation & Embryology Authority (HFEA).
187
188 A few tissue banks, repositories and resources to support research in human development are
189 available. For example, the UK's main fetal tissue bank, the Human Developmental Biology
190 Resource (HDBR: www.hdbr.org), jointly funded by MRC (now part of UK Research &
191 Innovation) and Wellcome, provides research material to UK and non-UK researchers. Non-UK
192 recipients of tissue must obtain their own project-specific ethics approval, prior to receipt of
193 material. This includes embryonic and fetal samples from 4 to 20 weeks post-fertilization with
194 karyotype information and, increasingly, with maternal DNA and clinical history information,
195 provided on an anonymised basis. Material from fetuses with prenatally diagnosed disorders (e.g.
196 trisomy 21) is also available. The French Human Developmental Cell Atlas (HuDeCA:
197 https://hudeca.genouest.org) biobank was recently established and funded by the public Institut
198 National de la Santé et de la Recherche Médicale (INSERM); HuDeCA also includes pre-
199 implantation embryos. It aspires to constitute the most comprehensive cohort in continental Europe
200 of human embryonic or early fetal samples, with strict quality control procedures and use of
201 standardized annotations, to further national and ultimately, international research projects, in
202 parallel with HDBR.
203
204 International sharing of genomic sequencing and clinical datasets derived from developmental and
205 pediatric tissue samples is subject to governing data protection regulation that considers
206 live/deceased status, consent regarding research data use, and the credibility of guarantees of
207 individual anonymity. Sensitive data, particularly from living donors, may need to be shared under

208 access controls, and be subject to appropriate privacy and security management frameworks. The
209 Human Cell Atlas (HCA) Ethics Working Group is currently developing a number of tools and
210 guidance notes (available at www.humancellatlas.org/ethics) including consent form templates
211 and sampling information for embryonic, fetal and pediatric tissue material, and international data
212 sharing guidance to support the developmental cell atlas community.
213
214 **Mapping development across space and time**
215 Development is intricately orchestrated in three spatial dimensions, with time as a fourth
216 dimension. Human embryogenesis cannot be assessed *in vivo* with the current resolution of
217 ultrasound technologies, nor is it amenable to intra-vital imaging through a surgical window as has
218 been applied to rodents [20]. Time-lapse studies are limited to pre-implantation stages where the
219 embryo is assessed *in vitro*. The application of high throughput genomics technologies to
220 dissociated cells and to tissue sections *in situ* is beginning to provide us with datasets of
221 unprecedented resolution to reconstruct human prenatal development (**Figure 3 and Table 1**).
222
223 *Cellular and molecular heterogeneity*
224 Single cell molecular profiles, such as for RNA, chromatin accessibility, or select protein
225 signatures have enabled a more nuanced definition of cell types and states based on
226 computationally driven models that determine reproducible characteristics and markers. The data
227 underpinning such definitions are increasingly derived from scRNA-seq and also accessible
228 chromatin sequencing of dissociated cells, with a range of robust, scalable and interrelatable
229 technologies [21]. Massively parallel methods, including droplet-based, microwell-, and
230 combinatorial-indexing approaches excel at profiling large numbers of cells for RNA [22], chromatin

231 [23], and proteins (with panels of DNA-barcoded antibodies) [24]. Resolving cell types and trajectories
232 at high granularity is aided by full-length scRNA-seq but primarily by profiling large numbers of
233 cells. A recent interesting development is the coupling of high-throughput tag-sequencing with
234 long-read technology to allow changes in gene splicing to be tracked over developmental time [25],
235 adding a further layer of information for cell type definition.
236
237 scRNA-seq and scATAC-Seq have also revealed molecular states and gene programs in these cell
238 types [26]. Development presents a greater challenge as cell type definition is currently guided by
239 existing knowledge of adult cellular profiles, which may or may not faithfully reflect prenatal cell
240 types, and the presence of transient cell types during development without a corresponding post-
241 natal counterpart. Furthermore, many cells will be in transitional states of differentiation during
242 development, with the cell states viewed as points along a continuum of developmental time and
243 space, rather than discrete entities. To overcome these challenges, many time points need to be
244 profiled, and defined cell states will need to be mapped back into their 3D space over time and
245 functionally characterised. High levels of multiplexing can attain this level of granularity at an
246 affordable cost for a complete developmental cell atlas [27,28].
247
248 Disentangling the relation between the overall state (and profile) of a cell, its discrete type, and
249 programs that reflect specific physiological features is one of the key open questions in the field.
250 Moreover, in addition to molecular profiles, other features, including morphology and functional
251 assessment can reflect the cell's state. Although these profiles emanate from the same cell, each
252 may reflect different facets. For example, among molecular features, the transcriptome reflects the
253 present and potential future of a cell whereas protein expression captures the immediate past and

254 present state of a cell, chromatin profiles can capture both its invariant type and potential for future
255 differentiation, and ontogeny reveals its history.
256
257 The field of developmental biology has traditionally drawn on ontogenic relationships to define
258 cell types, but this is challenging in humans, where information is often captured as a snapshot
259 series. Technologies, for example, CRISPR scarring as applied in model organisms is only
260 applicable in human organoid systems or preimplantation embryos for ethical reasons [29,30].
261 Inference of somatic mutations is the only available technology to definitively determine ontogeny,
262 but is limited by its current lack of scalability to analyse large cell numbers [31,32]. Recent methods
263 that rely on simultaneous measurement of mitochondrial DNA or RNA along with scATAC-Seq
264 or scRNA-Seq are poised to address this challenge [33,34].
265
266 We anticipate the field moving towards a consensus cell ontology that integrates multi-modal
267 single-cell profiling data (combined protein-transcriptome and chromatin-transcriptome profiling
268 e.g. CITE-seq, REAP-seq [35,36]; multi-parameter protein analysis e.g. mass cytometry, MICS,
269 CODEX, MIBI [37]; combinations of DNA modifications, chromatin accessibility and DNA
270 conformation [38] as well as legacy knowledge of embryonic cell type definitions augmented by
271 information from multiple animal models across evolutionary time. Multi-omics data sets from
272 identical cells will refine cell type definitions and function as a scaffold to align single modality
273 genomics datasets.
274
275
276

Mapping cells in 2D and 3D

There has been an explosion of spatial genomics methods to measure RNA molecules in tissue sections. These methods typically offer a trade-off between genomic scale and spatial resolution: methods with high resolution (cellular and subcellular) typically measure hundreds of genes, relying on RNA-capture for cDNA synthesis, rolling circle amplification and RNA-hybridisation technologies. Conversely, spatial transcriptomics methods capture RNA over 50 micron areas, but provide comprehensive molecular profiles [39–44]. This trade-off is often 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 measurements from lower resolution methods.

Tissue clearing methods to render organs transparent [45] combined with whole-mount protein immunostaining and RNA single-molecule FISH [46,47] can now provide 3D molecular profiling at cellular or subcellular resolution using light-sheet microscopy [48]. A 3D imaging pipeline applied to embryonic/fetal human organs and even whole human embryos [49–51] has proven its value in mapping cells during certain developmental stages. Progress is being made to increase the multiplex capacity of this approach and use of artificial intelligence/machine learning algorithms to overcome data analytical challenges, as was recently deployed to study whole-organismal vasculature following tissue clearing [52,53].

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 morphogenesis. Surface tension and pressure can be

300 measured in single cells and more recently in preimplantation mouse embryos [54]. Adapting these
301 technologies for human pre-implantation embryos and ES cell-derived embryo-like structures [55] is
302 anticipated to build a spatiotemporal mechanical atlas. Adoptive transfer of human iPS-derived
303 cells into the mouse, as was demonstrated for human iPS-derived neurons in the mouse brain [56],
304 provides new avenues for live imaging and functional mapping of developing human cells in a
305 complex and potentially relevant spatiotemporal context.
306
307 **Positional landmarks in development**
308 A standard coordinate system that describes locations in the human body (a common coordinate
309 framework (CCF)) is crucial for the Human Cell Atlas [57]. Two types of systems are useful:
310 absolute, similar to postcode/zip-code addresses, and relative, similar to a landmark-based address
311 system. Both types of systems usually require hierarchical organization. CCF anatomical
312 'postcodes' enable integration of multi-modal datasets of different spatial and longitudinal
313 resolution. The Allen Mouse Brain Reference Atlas v3 provides a CCF for the mouse brain,
314 containing anatomical features in 3D incorporating local features that are grouped in a hierarchy
315 to facilitate multilevel analysis. Efforts are currently underway to establish CCFs for adult human
316 organs within the NIH-HuBMAP initiative. The HDCA will need to develop a CCF that
317 incorporates space and time, as well as cell movement and patterns during organogenesis.
318 Integration with the Uberon (https://www.ebi.ac.uk/ols/ontologies/uberon) cell and developmental
319 structure ontology will facilitate the construction of developmental CCFs.
320
321
322

323 **Computation and data visualisation**

324 Given the challenges above, algorithms will play a key role in moving from a data collection to an
325 integrated atlas and model of development. Among the key algorithmic challenges are i) mapping
326 cells, which could be more fluid than discrete compared to adult counterparts; ii) inferring time
327 orderings, and lineage relations, including branching lineages, lineage potential and multiple
328 alternative paths converging on the same outcome (i.e. overcoming current limitations of fixed
329 hierarchies); iii) inferring spatial movement of cells; iv) building a temporal series of common
330 coordinates frameworks, with each being a probabilistic model for a particular time window as
331 well as a model for their morphing along time [58]; v) mapping across modalities and time points
332 (e.g. chromatin states in one time window to RNA and protein levels of another), and vi) inference
333 of regulatory and molecular networks within and across cells that drive these processes**.** The
334 HDCA community must also apply FAIR principles whenever possible to help ensure
335 reproducibility [59]. Integration of atlas data must support construction and updates of community
336 models that will use these time-ordered snapshots of spatially located molecular data for inferring
337 time-dependent processes including cell migration, cell lineage relationships, and gene regulatory
338 networks.

339

340 Following integrated atlas construction, how do we analyze the resulting models to move beyond
341 a phenomenology of the continuous and dynamic developmental process encompassing structural
342 and functional change over time and at multiple scales of space (molecule to cell to anatomical
343 structure)? New theories from multiple fields are required to delineate the mechanisms
344 underpinning canalization during tissue formation and growth [60]. A historically influential example
345 of this is Alan Turing's combination of biology, chemistry and mathematics to develop a theory

346 of morphogenesis and pattern formation in development [61]. It is likely that many additional
347 emergent properties of cells and their ecosystems will be discovered using such an interdisciplinary
348 approach and some of these will need new vocabularies, ontologies and modeling approaches to
349 uncover and understand. These approaches will need to consider vast and computable multi-omics
350 data, concurrently model state, position, internal and external factors and environment, and be able
351 to predict the state and 3D location of many components across time as development unfolds.
352
353 Computational integration of multi-omics data for 'Google maps'-like visualisation, such as the
354 Open Microscopy Environment (https://www.openmicroscopy.org/), will allow the user to zoom
355 into the single cell level from a large-volume tissue view. Additional complexity comes from
356 combining imaging and sequencing data together. In this case visualisations must link quantitative
357 information about gene expression, such as tSNE or UMAP plots of cells produced from the
358 sequencing RNA data, with a specific image. Sophisticated abstraction of the raw data and
359 integration across data modalities anchored by a developmental CCF, based on existing macro-
360 level 3D coordinates for human embryos, such as (http://hdbratlas.org/ and https://transparent-
361 human- embryo.com/), will be essential. The utility of the atlas data can be enhanced through links
362 to disease databases and ontologies, to broaden the ability to query the data with reference to
363 disease-related gene expression and cell localisation.
364
365 **Emerging cell atlases of human development**
366 Single cell suspension and spatial profiling of prenatal human organs have begun in recent years.
367 The advantages from whole tissue/organ profiling compared to selective cell type or lineage-
368 centric analysis include comprehensive analysis of all potential cell states, ability to discover

369 entities that are either entirely unknown or do not conform to pre-selected categorization, as well
370 as the ability to study interactions between different cells in one tissue microenvironment. This
371 strategy has already empowered our understanding of transient functional roles that organs may
372 perform during development. For example, the developing liver functions as a haematopoietic
373 organ during early gestation until mid second trimester, before it functionally transitions into a
374 metabolic organ similar to the adult liver [51]. To meet the high demand for erythropoiesis during
375 development, the first trimester human skin can also support erythrocyte maturation [51].
376
377 In stark contrast to our terrestrial postnatal life, the human embryo/fetus exists in an aquatic
378 environment. Our barrier organs, lung, gut and skin are exposed to amniotic fluid. In contrast to
379 postnatal lung, the developing lung does not perform oxygen transfer or receive the same volume
380 of blood through the pulmonary veins. The impact of these physiological factors on individual
381 tissues and the role of placenta and maternal decidua in supporting human embryogenesis and fetal
382 life are emerging [62,63].
383
384 Organ atlases of lung, heart, gastrointestinal tract, kidney, germ cells and gonads, and brain (**Table**
385 **1**) underscore the importance of studying human samples and reveal the unique aspects of human
386 development not conserved with animal model systems [64–66]. These include timelines of
387 development during gestation, cell type markers and expression pattern of transcription factors
388 between mouse and human organs [67,68].
389
390 The specification of functional tissue niches occurs during both prenatal and postnatal life. Fetal
391 gut studies highlight the importance of interactions between the epithelial and mesenchymal

392 compartments to allow the formation of villi and have identified fetal gut transcription factors that
393 are aberrantly activated in pediatric Crohn's disease [69]. Comparison between developing and adult
394 kidney demonstrated the establishment of dedicated spatial zonation against uropathogenic
395 bacterial challenge to occur only during postnatal life [70]. Single-cell transcriptomics of germ cells
396 during development have revealed important insights into the main pathways controlling their
397 differentiation [71,72] with ongoing studies focused on unravelling the regulatory mechanisms of sex
398 determination.
399
400 Early developmental studies of the brain have focused on human and primate cortical development
401 [73–82]. The developing human and rodent midbrain, which contains the clinically relevant
402 dopaminergic cell groups that are lost in Parkinson's disease, has also been extensively studied
403 [68,83,84], as has the developing mouse spinal cord and cerebellum [85,86], the hypothalamic arcuate
404 nucleus and the diencephalon [87].
405
406 Atlases of distributed systems such as the immune system have also been initiated, detailing their
407 generation in haematopoietic organs such as the fetal yolk sac and liver [51], lymphoid tissues such
408 as thymus where T cells differentiate [88] and non-lymphoid tissues such as skin and kidney where
409 immune cells reside. These studies revealed an intrinsic change in the differentiation potential of
410 haematopoietic stem cells/multipotent progenitor cells with gestational time together with the
411 importance of the tissue microenvironment for blood and immune cell development.
412
413
414

415 **Model organisms and culture systems**

416 As mentioned above, our understanding of human development has been largely inferred from
417 studies on animal model systems that have different developmental timelines and involve cellular
418 and molecular processes that are not always conserved across species (**Figure 1**) [89]. However, the
419 feasibility of perturbation and in depth mechanistic studies using animal models and culture
420 systems provide a valuable scaffold and complement the HDCA, particularly for the immediate
421 weeks after implantation where samples are inaccessible.

422

423 During the past five years, single cell molecular profiling has transformed many aspects of
424 developmental biology research across all major model organisms [90–94]. Because of the relative
425 ease of experimental manipulations, single cell studies in model organisms provide rapid means
426 of experimentally validating new hypotheses, and therefore are ideally placed to provide new
427 mechanistic insights into fundamental biological processes. Recent single cell studies have yielded
428 new biological insights into a variety of key processes, including the early specification of germ
429 layers and diversification of early cardiovascular cells [26,95].

430

431 Availability of parallel human and model species datasets will enable a whole host of cross-species
432 analyses. For example, computational analysis can connect cells in the human data with putative
433 developmental lineages reconstructed from time-resolved and experimentally validated model
434 organism data, can use a denser (model organisms) lineage to "fill in" a sparser human one, or can
435 align inferred lineages across two species or more. Identification of inter-species conservation as
436 well as divergence will facilitate accurate extrapolation of findings from non-primate models and
437 importantly inform the design of future animal models to serve as optimal surrogates for both

438 normal and pathological human development. Comparative studies of human and mouse pre-
439 implantation and gastrulation embryos indeed revealed conserved and divergent transcriptional
440 programs. For example, in the mouse KLF2 expression in embryo-fated epiblast progenitor cells
441 is not observed in humans; and by contrast, KLF17 is enriched in the human, but not mouse [96].
442 FGF8, required for mouse gastrulation, is not needed in the human embryo of the same stage [97].
443 Due to the availability of *in vivo* experimental systems to study lineage mapping in animal models,
444 comparative biology has the potential to make major contributions to one of the most pervasive
445 issues of single cell biology, namely cell ontology, including its relevance, utility and limitations.
446 Comparative lineage reconstruction also represents one of the more promising approaches to
447 connect human developmental datasets with those key early stages of mammalian development
448 that are largely inaccessible for human studies, such as the first few weeks after implantation.
449
450 Complex multicellular culture systems that accurately recapitulate human physiology can be used
451 to understand human development and model human disease. HPSC-derived organoids are
452 attractive systems because they can be genetically manipulated, observed in controlled *in vitro*
453 environments, and derived from individuals with diverse genetic backgrounds [98]. Organoid
454 generation relies on directed differentiation, a process that uses temporal manipulation of key
455 developmental signaling pathways via exogenously supplied growth factors and small molecules
456 to mimic organogenesis in a step-by-step manner [99,100]. HPSC-derived organoids can therefore
457 recapitulate many aspects of organ specification and complex tissue formation that occur during
458 very early stages of development. Conversely, analyzing native human samples can identify new
459 secreted factors to promote development of more faithful organoids.
460

461 Self-organization of human embryonic tissue can be captured from the earliest moments *in vitro*
462 [101], and extended to gastrulation, anterior-posterior embryonic patterning, and early phases of
463 somitogenesis [102]. The recent human gastrulation embryo dataset will be informative as a
464 benchmark to further refine *in vitro* directed differentiation of human cells, including gastruloid
465 models [102], to more faithfully recapitulate early *in vivo* differentiated cell types and gastrulation.
466 Other processes during organogenesis can also be monitored, including clock control of somite
467 segmentation [103], boundary formations during hepato-biliary-pancreatic organ budding [104] and
468 patterning of the neural tube. Protocols are now established to mimic development of diverse
469 human tissues that exhibit morphologies and physiologic functionalities of developing human
470 tissues. Such organoid systems include hair-bearing skin [105]; small intestine with a crypt-villus
471 axis [98]; region-specific [106] and multi-region [107] brain tissue modeling neurogenesis, neural
472 migration, and synapse formation; multi-layered neural retina with photoreception responses [108];
473 arterio-venous specification during blood vessel development [109], and many other human tissues.
474
475 Cell composition within some of these organoid systems have already been analyzed using single-
476 cell transcriptomics [107]. However, a comprehensive reference atlas of cell types and states present
477 during human development will be critical to benchmark stem cell-derived organoids. Such
478 roadmap comparisons will highlight similarities [80], illuminate deficiencies [110], and define strategies
479 for improving organoids for disease modeling. In the future, high-fidelity hPSC-derived human
480 organoids and single-cell multi-omic modalities will be extraordinarily powerful tools to
481 understand the mechanisms that control the unique features of human organogenesis.
482
483

484 **Clinical relevance and applications of a human developmental cell atlas**

485 There is mounting evidence that disorders identified in childhood and adulthood may have their
486 origins and manifestations during early development (**Figure 4**). These include structural birth
487 defects [111–114], neurodevelopmental disorders including Huntington's disease [115], childhood
488 cancers [70]; [2]; [116], inborn errors of immunity [117], infertility and differences of sex development [118]
489 [119] as well as many pediatric disorders [120]. Developmental perturbations can also give rise to
490 complex diseases manifesting in adult life e.g. Down syndrome (trisomy 21) [121] and 22q11.2
491 deletion syndrome [122] that present a spectrum of perturbed developmental sequelae at birth and
492 significant risks for schizophrenia [123], Alzheimer's disease, and hypothyroidism in later life [123].
493 Moreover, many adult cancers recapitulate a grotesque version of human developmental programs
494 [124]. Conversely, molecular processes that guide human development can be understood by studying
495 developmental disorders [125], many with significant individual and public health impacts.
496 Identifying the etiology of developmental disorders and the effect of external factors such as diet,
497 alcohol, toxins, endocrine disruptors and pathogens on development have been hampered by our
498 limited understanding of normal human development.

499

500 Development atlases are unravelling the pathogenesis of childhood cancers (**Figure 4**). Pediatric
501 and adult brain tumors in their early stages often present impaired developmental programs within
502 tumor cells [126,127]. A single-cell atlas of the developing mouse cerebellum was used to dissect
503 subtypes of human medulloblastoma, a pediatric brain tumor [2,116]. Comparing the expression
504 profile of tumor cells with HDCA can identify the cancer cell of origin and its oncogenic pathways.
505 For example, cell states during nephrogenesis discerned the developmental cellular origin of

506 Wilms tumour [70]. High resolution mapping of developing immune cells will inform the molecular
507 and extent of disease phenotypes of childhood leukaemias and primary immunodeficiencies.
508
509 Cell and tissue engineering for clinical therapies and regenerative medicine are areas with
510 enormous potential for the direct utility and applications emerging from the detailed molecular
511 information contained within the HDCA. Haematopoietic stem cell (HSC) transplantation is an
512 established and widely used treatment for many haematological and increasingly non-
513 haematological disorders. Leveraging the potency factors of fetal HSCs can have significant
514 benefit to patients. The same principle will instruct adult stem cell regeneration applications for
515 enhancing health and in clinical therapy.
516
517 **Towards a whole embryo atlas**
518 The initial HCA White paper emphasized 12 distinct organ systems within the human body, and
519 highlighted the importance of a developmental cell atlas. Integrated multi-organ analyses will
520 provide novel insights on tissue microenvironment shaping resident epithelial, stroma and immune
521 cells and the cellular heterogeneity of innervating blood vessels, lymphatics, and peripheral nerves.
522 Eventually, this may illuminate system level lineage development and cell fate decision across an
523 entire organism.
524
525 There are several large scale organ-based ongoing studies. These include NIH BRAIN Initiative
526 BICCN consortium focusing on the developing human cortex, the Swedish HCA consortium
527 performing large-scale single-cell RNA-seq, ATAC-seq and spatial-omic analysis of the
528 developing human brain, the French HuDeCA consortium to map the development of eight human

529 organs using 3D imaging and scRNA-seq during the first trimester of gestation, EU H2020-funded
530 developing brain (Braintime) and gonad (Hugodeca: [128] https://hugodeca-project.eu/), the NIH
531 Developmental Genotype-Tissue Expression (dGTEx) initiative
532 (https://www.genome.gov/Funded-Programs-Projects/Developmental-Genotype-Tissue-
533 Expression) and Wellcome and MRC-funded consortia in the UK. The logical next step will be to
534 extend the current approach step by step to contextualise the development of different cell lineages
535 across all organs.
536
537 Importantly, multi-organ approaches do not permit the analysis of organs with their connecting
538 structures and distributed networks as a continuum from one donor sample. Furthermore, the
539 impact of the embryonic hormone and growth factor milieu that act in distant organs is not easily
540 evaluated by multi-organ analysis. How can we decipher the coordinated development *in toto* for
541 the entire human organism? At the current stage, whole embryo analysis has been limited to very
542 early pre-implantation samples [96,129,130] and one gastrulation stage embryo [97]. Whole embryos and
543 fetuses have been analysed by light sheet imaging [50] but present significant challenges for genomic
544 technologies due to scale of cell numbers and size of samples to be profiled. Multi-omics
545 suspension and spatial profiling of anatomically dissected units with careful consideration of
546 specific tissue and cell type/state coverage is one approach that will be attempted by the UK HDCA
547 consortium. Micro level analysis can be combined with macro scale analysis to provide breadth
548 and depth of the atlas. Multi-omics data integration must discern batch and tissue processing
549 artefacts from true biological changes across tissue and over gestation time.
550

551 In conclusion, a complete whole embryo-fetal cell atlas across human gestation will be challenging
552 but possible in the near future. It will require a global collaborative multi-disciplinary team effort,
553 multiple experimental and technological approaches, as well as inferences from *in vitro* culture
554 systems and model organisms. The HDCA will be a transformative resource for the research and
555 clinical communities.
556
557 **Figure and table legends**
558
559 Figure 1: Milestones in human developmental research and model systems
560 a. Milestones in human developmental research including new technologies, publications and
561 scientific break-throughs.
562 b. *In vitro* model systems to study early embryonic development.
563 c. Experimental model systems to study development, including *D. melanogaster*, *D. rerio*,
564 *X. laevis*, *M. musculus*, cell culture and organoids, and their amenability to facilitate
565 various aspects of scientific study.
566
567 Figure 2: The Human Developmental Cell Atlas: how to build it and what will it provide?
568
569 a. 'How to build an atlas' modules, including an interdisciplinary team, multi-modal
570 technologies, and integration of data across platforms.
571 b. Key features of the Human Development Cell Atlas. Single cell measurements across three
572 dimensional space, alongside a fourth dimension of time, allow for capture of dynamic
573 developmental processes including cell proliferation, migration and regulation.

574 c. Utility and applications of the Human Development Cell Atlas: cellular and molecular
575 biological insights applied to advance regenerative medicine, tissue engineering and
576 therapeutics.
577
578 Figure 3: Multi-omics profiling and data integration
579
580 a. Organ or anatomical unit profiling of a prenatal embryo derived from the three germ layers.
581 b. Single cell atlas technologies by relative resolution and genome scale.
582 c. Integration of datasets from different technologies (e.g., spatial transcriptomics, single-cell
583 RNA sequencing, targeted *in situ* sequencing) to profile organs or whole embryo.
584
585 Figure 4: Clinical relevance and applications of the Human Developmental Cell Atlas
586
587 a. A timeline of brain development across human life, with examples of diseases with onset
588 at different gestational stages and ages.
589 b. How a single cell atlas with temporal and spatial information can be used as a reference to
590 understand disease.
591
592 Table 1: Publications from the Human Development Cell Atlas initiative and their highlights.
593
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Conflict of interest

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641 **References**

642 1. Behjati, S., Lindsay, S., Teichmann, S. A. & Haniffa, M. Mapping human development at
643 single-cell resolution. *Development* **145**, (2018).

644 2. Vladoiu, M. C. *et al.* Childhood cerebellar tumours mirror conserved fetal transcriptional
645 programs. *Nature* **572**, 67–73 (2019).

646 3. Waardenberg, A. J., Ramialison, M., Bouveret, R. & Harvey, R. P. Genetic networks
647 governing heart development. *Cold Spring Harb. Perspect. Med.* **4**, a013839 (2014).

648 4. Velmeshev, D. *et al.* Single-cell genomics identifies cell type-specific molecular changes in
649 autism. *Science* **364**, 685–689 (2019).

650 5. Gulsuner, S. *et al.* Spatial and temporal mapping of de novo mutations in schizophrenia to a
651 fetal prefrontal cortical network. *Cell* **154**, 518–529 (2013).

652 6. Simmons, R. A. Developmental origins of adult disease. *Pediatr. Clin. North Am.* **56**, 449–
653 66, Table of Contents (2009).

654 7. Laughney, A. M. *et al.* Regenerative lineages and immune-mediated pruning in lung cancer
655 metastasis. *Nat. Med.* **26**, 259–269 (2020).

656 8. Camp, J. G., Wollny, D. & Treutlein, B. Single-cell genomics to guide human stem cell and
657 tissue engineering. *Nat. Methods* **15**, 661–667 (2018).

658 9. Morgan, L. *Icons of Life: A Cultural History of Human Embryos*. (University of California
659 Press, 2009).

660 10. Blonder, L. X. Morphogenesis: The cellular and molecular processes of developmental
661 anatomy. By Jonathan Bard. xi 313 pp. New York: Cambridge University Press. 1990.
662 $37.95. (paper). *American Journal of Human Biology* vol. 5 245–246 (1993).

663 11. Aldridge, S. & Teichmann, S. A. Single cell transcriptomics comes of age. *Nat. Commun.* **11**,

664 4307 (2020).

665 12. Bendall, S. C. *et al.* Single-cell trajectory detection uncovers progression and regulatory
666 coordination in human B cell development. *Cell* **157**, 714–725 (2014).

667 13. Haghverdi, L., Büttner, M., Wolf, F. A., Buettner, F. & Theis, F. J. Diffusion pseudotime
668 robustly reconstructs lineage branching. *Nat. Methods* **13**, 845–848 (2016).

669 14. Asp, M. *et al.* A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the
670 Developing Human Heart. *Cell* **179**, 1647–1660.e19 (2019).

671 15. Regev, A., Teichmann, S. A., Lander, E. S., Amit, I. & Benoist, C. Science forum: the human
672 cell atlas. *Elife* **6**, e27041 (2017).

673 16. Parker, H. J., Pushel, I. & Krumlauf, R. Coupling the roles of Hox genes to regulatory
674 networks patterning cranial neural crest. *Dev. Biol.* **444 Suppl 1**, S67–S78 (2018).

675 17. Chin, A. M., Hill, D. R., Aurora, M. & Spence, J. R. Morphogenesis and maturation of the
676 embryonic and postnatal intestine. *Semin. Cell Dev. Biol.* **66**, 81–93 (2017).

677 18. Morrisey, E. E. & Hogan, B. L. M. Preparing for the first breath: genetic and cellular
678 mechanisms in lung development. *Dev. Cell* **18**, 8–23 (2010).

679 19. van der Lee, R., Correard, S. & Wasserman, W. W. Deregulated Regulators: Disease-Causing
680 cis Variants in Transcription Factor Genes. *Trends Genet.* **36**, 523–539 (2020).

681 20. Huang, Q. *et al.* Intravital imaging of mouse embryos. *Science* vol. 368 181–186 (2020).

682 21. Mereu, E. *et al.* Benchmarking single-cell RNA-sequencing protocols for cell atlas projects.
683 *Nat. Biotechnol.* **38**, 747–755 (2020).

684 22. Vitak, S. A. *et al.* Sequencing thousands of single-cell genomes with combinatorial indexing.
685 *Nat. Methods* **14**, 302–308 (2017).

686 23. Lareau, C. A. *et al.* Droplet-based combinatorial indexing for massive-scale single-cell

687 chromatin accessibility. *Nat. Biotechnol.* **37**, 916–924 (2019).

688 24. Stoeckius, M. *et al.* Simultaneous epitope and transcriptome measurement in single cells. *Nat.*
689 *Methods* **14**, 865–868 (2017).

690 25. Lebrigand, K., Magnone, V., Barbry, P. & Waldmann, R. High throughput, error corrected
691 Nanopore single cell transcriptome sequencing. (2020) doi:10.1101/831495.

692 26. Argelaguet, R. *et al.* Multi-omics profiling of mouse gastrulation at single-cell resolution.
693 *Nature* **576**, 487–491 (2019).

694 27. Cao, J. *et al.* Comprehensive single-cell transcriptional profiling of a multicellular organism.
695 *Science* **357**, 661–667 (2017).

696 28. McGinnis, C. S. *et al.* MULTI-seq: sample multiplexing for single-cell RNA sequencing
697 using lipid-tagged indices. *Nat. Methods* **16**, 619–626 (2019).

698 29. Fujii, M., Clevers, H. & Sato, T. Modeling Human Digestive Diseases With CRISPR-Cas9–
699 Modified Organoids. *Gastroenterology* vol. 156 562–576 (2019).

700 30. Artegiani, B. *et al.* Fast and efficient generation of knock-in human organoids using
701 homology-independent CRISPR-Cas9 precision genome editing. *Nat. Cell Biol.* **22**, 321–331
702 (2020).

703 31. Lee-Six, H. *et al.* Population dynamics of normal human blood inferred from somatic
704 mutations. *Nature* **561**, 473–478 (2018).

705 32. D'Gama, A. M. & Walsh, C. A. Somatic mosaicism and neurodevelopmental disease. *Nat.*
706 *Neurosci.* **21**, 1504–1514 (2018).

707 33. Ludwig, L. S. *et al.* Lineage Tracing in Humans Enabled by Mitochondrial Mutations and
708 Single-Cell Genomics. *Cell* **176**, 1325–1339.e22 (2019).

709 34. Lareau, C. A. *et al.* Massively parallel single-cell mitochondrial DNA genotyping and

710 chromatin profiling. *Nat. Biotechnol.* (2020) doi:10.1038/s41587-020-0645-6.

711 35. Mimitou, E. P. *et al.* Multiplexed detection of proteins, transcriptomes, clonotypes and
712 CRISPR perturbations in single cells. *Nat. Methods* **16**, 409–412 (2019).

713 36. Peterson, V. M. *et al.* Multiplexed quantification of proteins and transcripts in single cells.
714 *Nat. Biotechnol.* **35**, 936–939 (2017).

715 37. Labib, M. & Kelley, S. O. Single-cell analysis targeting the proteome. *Nature Reviews*
716 *Chemistry* vol. 4 143–158 (2020).

717 38. Gu, C., Liu, S., Wu, Q., Zhang, L. & Guo, F. Integrative single-cell analysis of transcriptome,
718 DNA methylome and chromatin accessibility in mouse oocytes. *Cell Research* vol. 29 110–
719 123 (2019).

720 39. Ståhl, P. L. *et al.* Visualization and analysis of gene expression in tissue sections by spatial
721 transcriptomics. *Science* **353**, 78–82 (2016).

722 40. Rodriques, S. G. *et al.* Slide-seq: A scalable technology for measuring genome-wide
723 expression at high spatial resolution. *Science* **363**, 1463–1467 (2019).

724 41. Chen, K. H., Boettiger, A. N., Moffitt, J. R., Wang, S. & Zhuang, X. Spatially resolved, highly
725 multiplexed RNA profiling in single cells. *Science* vol. 348 aaa6090–aaa6090 (2015).

726 42. Wang, X. *et al.* Three-dimensional intact-tissue sequencing of single-cell transcriptional
727 states. *Science* **361**, (2018).

728 43. Lubeck, E., Coskun, A. F., Zhiyentayev, T., Ahmad, M. & Cai, L. Single-cell in situ RNA
729 profiling by sequential hybridization. *Nature methods* vol. 11 360–361 (2014).

730 44. Ke, R. *et al.* In situ sequencing for RNA analysis in preserved tissue and cells. *Nat. Methods*
731 **10**, 857–860 (2013).

732 45. Ueda, H. R. *et al.* Tissue clearing and its applications in neuroscience. *Nat. Rev. Neurosci.*

733 **21**, 61–79 (2020).

734 46. Yang, B. *et al.* Single-cell phenotyping within transparent intact tissue through whole-body
735 clearing. *Cell* **158**, 945–958 (2014).

736 47. Sylwestrak, E. L., Rajasethupathy, P., Wright, M. A., Jaffe, A. & Deisseroth, K. Multiplexed
737 Intact-Tissue Transcriptional Analysis at Cellular Resolution. *Cell* **164**, 792–804 (2016).

738 48. Zhao, S. *et al.* Cellular and Molecular Probing of Intact Human Organs. *Cell* **180**, 796–
739 812.e19 (2020).

740 49. Casoni, F. *et al.* Development of the neurons controlling fertility in humans: new insights
741 from 3D imaging and transparent fetal brains. *Development* **143**, 3969–3981 (2016).

742 50. Belle, M. *et al.* Tridimensional Visualization and Analysis of Early Human Development.
743 *Cell* vol. 169 161–173.e12 (2017).

744 51. Popescu, D.-M. *et al.* Decoding human fetal liver haematopoiesis. *Nature* **574**, 365–371
745 (2019).

746 52. Todorov, M. I. *et al.* Machine learning analysis of whole mouse brain vasculature. *Nat.*
747 *Methods* **17**, 442–449 (2020).

748 53. Kirst, C. *et al.* Mapping the Fine-Scale Organization and Plasticity of the Brain Vasculature.
749 *Cell* **180**, 780–795.e25 (2020).

750 54. Dumortier, J. G. *et al.* Hydraulic fracturing and active coarsening position the lumen of the
751 mouse blastocyst. *Science* **365**, 465–468 (2019).

752 55. Shahbazi, M. N., Siggia, E. D. & Zernicka-Goetz, M. Self-organization of stem cells into
753 embryos: A window on early mammalian development. *Science* **364**, 948–951 (2019).

754 56. Linaro, D. *et al.* Xenotransplanted Human Cortical Neurons Reveal Species-Specific
755 Development and Functional Integration into Mouse Visual Circuits. *Neuron* **104**, 972–

986.e6 (2019).

57. Rood, J. E. *et al.* Toward a Common Coordinate Framework for the Human Body. *Cell* vol. 179 1455–1467 (2019).

58. Bonneel, N. Optimal Transport for Computer Graphics and Temporal Coherence of Image Processing Algorithms. (hal.sorbonne-universite.fr, 2018).

59. Wilkinson, M. D. *et al.* The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data* **3**, 160018 (2016).

60. Wong, M. & Gilmour, D. Getting back on track: exploiting canalization to uncover the mechanisms of developmental robustness. *Curr. Opin. Genet. Dev.* **63**, 53–60 (2020).

61. Turing, A. M. The chemical basis of morphogenesis. 1953. *Bull. Math. Biol.* **52**, 153–97; discussion 119–52 (1990).

62. Vento-Tormo, R. *et al.* Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* **563**, 347–353 (2018).

63. Suryawanshi, H. *et al.* A single-cell survey of the human first-trimester placenta and decidua. *Science Advances* vol. 4 eaau4788 (2018).

64. Czerwinski, M. *et al.* In vitro and in vivo development of the human intestinal niche at single cell resolution. doi:10.1101/2020.01.31.928788.

65. Pollen, A. A. *et al.* Low-coverage single-cell mRNA sequencing reveals cellular heterogeneity and activated signaling pathways in developing cerebral cortex. *Nature Biotechnology* vol. 32 1053–1058 (2014).

66. Han, X. *et al.* Construction of a human cell landscape at single-cell level. *Nature* **581**, 303–309 (2020).

67. Cui, Y. *et al.* Single-Cell Transcriptome Analysis Maps the Developmental Track of the

779 Human Heart. *Cell Reports* vol. 26 1934–1950.e5 (2019).

780 68. La Manno, G. *et al.* Molecular Diversity of Midbrain Development in Mouse, Human, and
781 Stem Cells. *Cell* **167**, 566–580.e19 (2016).

782 69. Elmentaite, R., Ross, A., James, K. R., Ortmann, D. & Gomes, T. Single-cell sequencing of
783 developing human gut reveals transcriptional links to childhood Crohns disease. *bioRxiv*
784 (2020).

785 70. Young, M. D. *et al.* Single-cell transcriptomes from human kidneys reveal the cellular identity
786 of renal tumors. *Science* **361**, 594–599 (2018).

787 71. Vértesy, Á. *et al.* Parental haplotype-specific single-cell transcriptomics reveal incomplete
788 epigenetic reprogramming in human female germ cells. *Nat. Commun.* **9**, 1873 (2018).

789 72. Li, L. *et al.* Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells
790 and Gonadal Niche Interactions. *Cell Stem Cell* **20**, 858–873.e4 (2017).

791 73. Johnson, M. B. & Walsh, C. A. Cerebral cortical neuron diversity and development at single-
792 cell resolution. *Curr. Opin. Neurobiol.* **42**, 9–16 (2017).

793 74. Nowakowski, T. J. *et al.* Spatiotemporal gene expression trajectories reveal developmental
794 hierarchies of the human cortex. *Science* **358**, 1318–1323 (2017).

795 75. Polioudakis, D. *et al.* A Single-Cell Transcriptomic Atlas of Human Neocortical
796 Development during Mid-gestation. *Neuron* **103**, 785–801.e8 (2019).

797 76. Lu, Y. *et al.* Single-Cell Analysis of Human Retina Identifies Evolutionarily Conserved and
798 Species-Specific Mechanisms Controlling Development. *Dev. Cell* **53**, 473–491.e9 (2020).

799 77. Haldipur, P. *et al.* Spatiotemporal expansion of primary progenitor zones in the developing
800 human cerebellum. *Science* **366**, 454–460 (2019).

801 78. Zhong, S. *et al.* A single-cell RNA-seq survey of the developmental landscape of the human

802 prefrontal cortex. *Nature* (2018).

803 79. Pollen, A. A., Nowakowski, T. J., Chen, J. & Retallack, H. Molecular identity of human outer
804 radial glia during cortical development. *Cell* (2015).

805 80. Camp, J. G. *et al.* Human cerebral organoids recapitulate gene expression programs of fetal
806 neocortex development. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 15672–15677 (2015).

807 81. Mayer, C., Hafemeister, C., Bandler, R. C. & Machold, R. Developmental diversification of
808 cortical inhibitory interneurons. *Nature* (2018).

809 82. Mi, D. *et al.* Early emergence of cortical interneuron diversity in the mouse embryo. (2018).

810 83. Tiklová, K. *et al.* Single-cell RNA sequencing reveals midbrain dopamine neuron diversity
811 emerging during mouse brain development. *Nat. Commun.* **10**, 581 (2019).

812 84. Kee, N. *et al.* Single-Cell Analysis Reveals a Close Relationship between Differentiating
813 Dopamine and Subthalamic Nucleus Neuronal Lineages. *Cell Stem Cell* **20**, 29–40 (2017).

814 85. Rosenberg, A. B., Roco, C. M., Muscat, R. A. & Kuchina, A. Single-cell profiling of the
815 developing mouse brain and spinal cord with split-pool barcoding. (2018).

816 86. Carter, R. A., Bihannic, L., Rosencrance, C. & Hadley, J. L. A single-cell transcriptional atlas
817 of the developing murine cerebellum. *Curr. Biol.* (2018).

818 87. Huisman, C. *et al.* Single cell transcriptome analysis of developing arcuate nucleus neurons
819 uncovers their key developmental regulators. *Nat. Commun.* **10**, 1–12 (2019).

820 88. Park, J.-E. *et al.* A cell atlas of human thymic development defines T cell repertoire formation.
821 *Science* **367**, (2020).

822 89. Rossant, J. & Tam, P. P. L. New Insights into Early Human Development: Lessons for Stem
823 Cell Derivation and Differentiation. *Cell Stem Cell* **20**, 18–28 (2017).

824 90. Pijuan-Sala, B. *et al.* A single-cell molecular map of mouse gastrulation and early

825 organogenesis. *Nature* **566**, 490–495 (2019).

826 91. Wagner, D. E. *et al.* Single-cell mapping of gene expression landscapes and lineage in the
827 zebrafish embryo. *Science* **360**, 981–987 (2018).

828 92. Briggs, J. A. *et al.* The dynamics of gene expression in vertebrate embryogenesis at single-
829 cell resolution. *Science* **360**, (2018).

830 93. Cao, J. *et al.* The single-cell transcriptional landscape of mammalian organogenesis. *Nature*
831 **566**, 496–502 (2019).

832 94. Cusanovich, D. A. *et al.* The cis-regulatory dynamics of embryonic development at single-
833 cell resolution. *Nature* **555**, 538–542 (2018).

834 95. Lescroart, F. *et al.* Defining the earliest step of cardiovascular lineage segregation by single-
835 cell RNA-seq. *Science* **359**, 1177–1181 (2018).

836 96. Blakeley, P. *et al.* Defining the three cell lineages of the human blastocyst by single-cell
837 RNA-seq. *Development* **142**, 3151–3165 (2015).

838 97. Tyser, R. C. V., Mahammadov, E., Nakanoh, S. & Vallier, L. A spatially resolved single cell
839 atlas of human gastrulation. *bioRxiv* (2020).

840 98. Spence, J. R. *et al.* Directed differentiation of human pluripotent stem cells into intestinal
841 tissue in vitro. *Nature* **470**, 105–109 (2011).

842 99. Zorn, A. M. & Wells, J. M. Vertebrate endoderm development and organ formation. *Annu.*
843 *Rev. Cell Dev. Biol.* **25**, 221–251 (2009).

844 100. McCracken, K. W., Howell, J. C., Wells, J. M. & Spence, J. R. Generating human intestinal
845 tissue from pluripotent stem cells in vitro. *Nat. Protoc.* **6**, 1920–1928 (2011).

846 101. Deglincerti, A. *et al.* Self-organization of the in vitro attached human embryo. *Nature* **533**,
847 251–254 (2016).

848 102. Moris, N. *et al.* An in vitro model of early anteroposterior organization during human
849 development. *Nature* **582**, 410–415 (2020).
850 103. Matsuda, M. *et al.* Recapitulating the human segmentation clock with pluripotent stem cells.
851 *Nature* **580**, 124–129 (2020).
852 104. Koike, H. *et al.* Modelling human hepato-biliary-pancreatic organogenesis from the foregut-
853 midgut boundary. *Nature* **574**, 112–116 (2019).
854 105. Lee, J. *et al.* Hair-bearing human skin generated entirely from pluripotent stem cells. *Nature*
855 **582**, 399–404 (2020).
856 106. Marton, R. M. & Pașca, S. P. Organoid and Assembloid Technologies for Investigating
857 Cellular Crosstalk in Human Brain Development and Disease. *Trends Cell Biol.* **30**, 133–143
858 (2020).
859 107. Lancaster, M. A. *et al.* Cerebral organoids model human brain development and
860 microcephaly. *Nature* **501**, 373–379 (2013).
861 108. Quadrato, G. *et al.* Cell diversity and network dynamics in photosensitive human brain
862 organoids. *Nature* **545**, 48–53 (2017).
863 109. Wimmer, R. A. *et al.* Human blood vessel organoids as a model of diabetic vasculopathy.
864 *Nature* **565**, 505–510 (2019).
865 110. Bhaduri, A. *et al.* Cell stress in cortical organoids impairs molecular subtype specification.
866 *Nature* **578**, 142–148 (2020).
867 111. Homsy, J. *et al.* De novo mutations in congenital heart disease with neurodevelopmental and
868 other congenital anomalies. *Science* **350**, 1262–1266 (2015).
869 112. Jin, S. C. *et al.* Contribution of rare inherited and de novo variants in 2,871 congenital heart
870 disease probands. *Nat. Genet.* **49**, 1593–1601 (2017).

871 113. Qi, H. *et al.* De novo variants in congenital diaphragmatic hernia identify MYRF as a new
872 syndrome and reveal genetic overlaps with other developmental disorders. *PLoS Genet.* **14**,
873 e1007822 (2018).
874 114. Mukhopadhyay, N. *et al.* Whole genome sequencing of orofacial cleft trios from the Gabriella
875 Miller Kids First Pediatric Research Consortium identifies a new locus on chromosome 21.
876 *Hum. Genet.* **139**, 215–226 (2020).
877 115. Barnat, M. *et al.* Huntington's disease alters human neurodevelopment. *Science* eaax3338
878 (2020) doi:10.1126/science.aax3338.
879 116. Hovestadt, V. *et al.* Medulloblastomics revisited: biological and clinical insights from
880 thousands of patients. *Nat. Rev. Cancer* **20**, 42–56 (2020).
881 117. Zhang, S.-Y. *et al.* Human inborn errors of immunity to infection affecting cells other than
882 leukocytes: from the immune system to the whole organism. *Curr. Opin. Immunol.* **59**, 88–
883 100 (2019).
884 118. Croft, B. *et al.* Human sex reversal is caused by duplication or deletion of core enhancers
885 upstream of SOX9. *Nat. Commun.* **9**, 5319 (2018).
886 119. Croft, B. *et al.* Author Correction: Human sex reversal is caused by duplication or deletion
887 of core enhancers upstream of SOX9. *Nat. Commun.* **10**, 3351 (2019).
888 120. The Pediatric Cell Atlas: Defining the Growth Phase of Human Development at Single-Cell
889 Resolution. *Dev. Cell* **49**, 10–29 (2019).
890 121. Ly, A. *et al.* DSCAM is a netrin receptor that collaborates with DCC in mediating turning
891 responses to netrin-1. *Cell* **133**, 1241–1254 (2008).
892 122. Yamagishi, H. & Srivastava, D. Unraveling the genetic and developmental mysteries of
893 22q11 deletion syndrome. *Trends Mol. Med.* **9**, 383–389 (2003).

894 123. Biswas, A. B. & Furniss, F. Cognitive phenotype and psychiatric disorder in 22q11.2 deletion
895 syndrome: A review. *Res. Dev. Disabil.* **53-54**, 242–257 (2016).
896 124. Phillips, H. S. *et al.* Molecular subclasses of high-grade glioma predict prognosis, delineate
897 a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* **9**, 157–
898 173 (2006).
899 125. Herion, N. J., Salbaum, J. M. & Kappen, C. Traffic jam in the primitive streak: the role of
900 defective mesoderm migration in birth defects. *Birth Defects Res. A Clin. Mol. Teratol.* **100**,
901 608–622 (2014).
902 126. Jessa, S. *et al.* Stalled developmental programs at the root of pediatric brain tumors. *Nat.*
903 *Genet.* **51**, 1702–1713 (2019).
904 127. Tirosh, I. *et al.* Single-cell RNA-seq supports a developmental hierarchy in human
905 oligodendroglioma. *Nature* **539**, 309–313 (2016).
906 128. Home - Hugodeca. https://hugodeca-project.eu/.
907 129. Yan, L. *et al.* Single-cell RNA-Seq profiling of human preimplantation embryos and
908 embryonic stem cells. *Nat. Struct. Mol. Biol.* **20**, 1131–1139 (2013).
909 130. Petropoulos, S. *et al.* Single-Cell RNA-Seq Reveals Lineage and X Chromosome Dynamics
910 in Human Preimplantation Embryos. *Cell* **167**, 285 (2016).
911

Figures

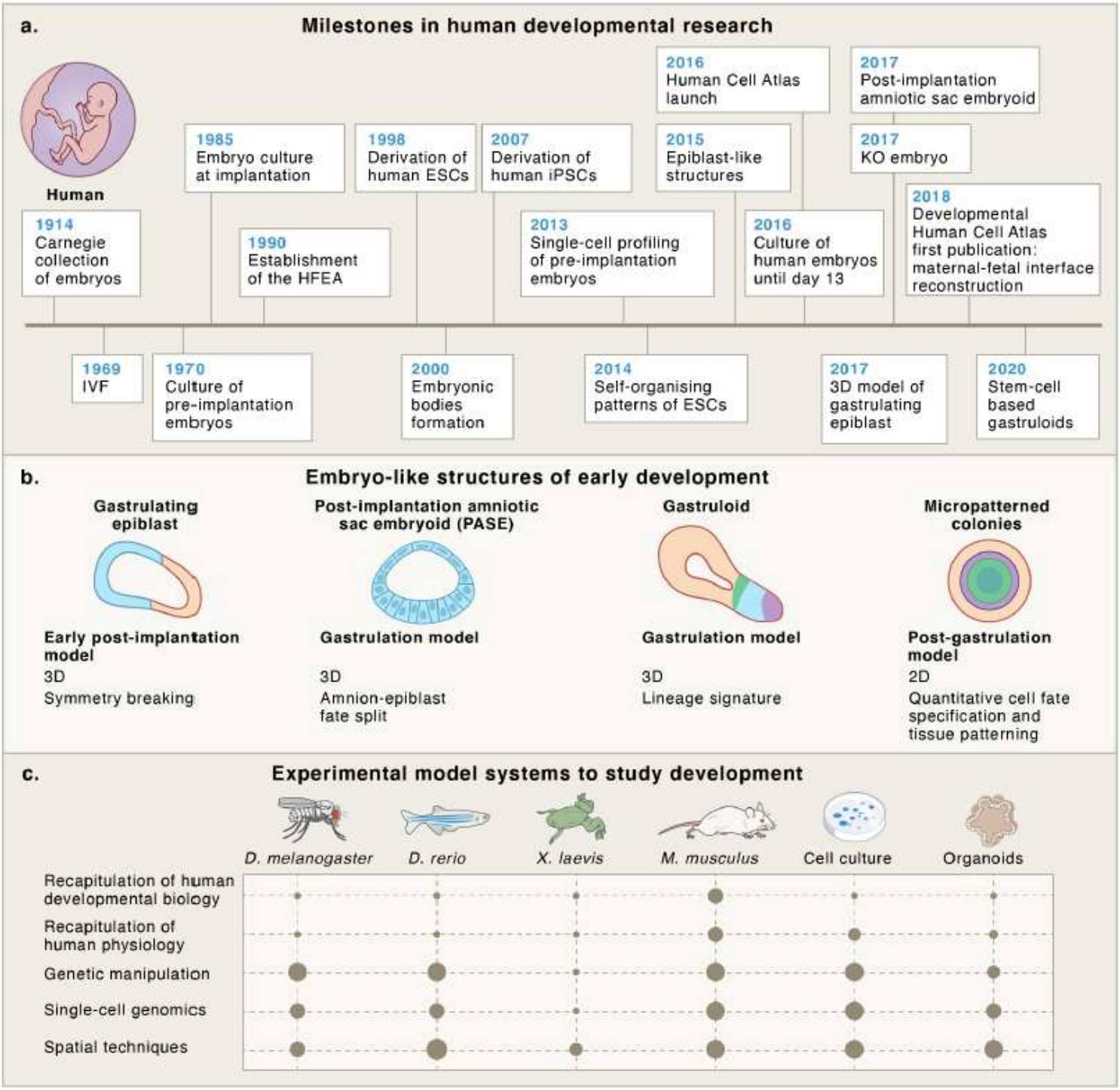

Figure 1

Milestones in human developmental research and model systems a. Milestones in human developmental research including new technologies, publications and scientific break-throughs. b. In vitro model systems to study early embryonic development. c. Experimental model systems to study development, including D. melanogaster, D. rerio, X. laevis, M. musculus, cell culture and organoids, and their amenability to facilitate 565 various aspects of scientific study.

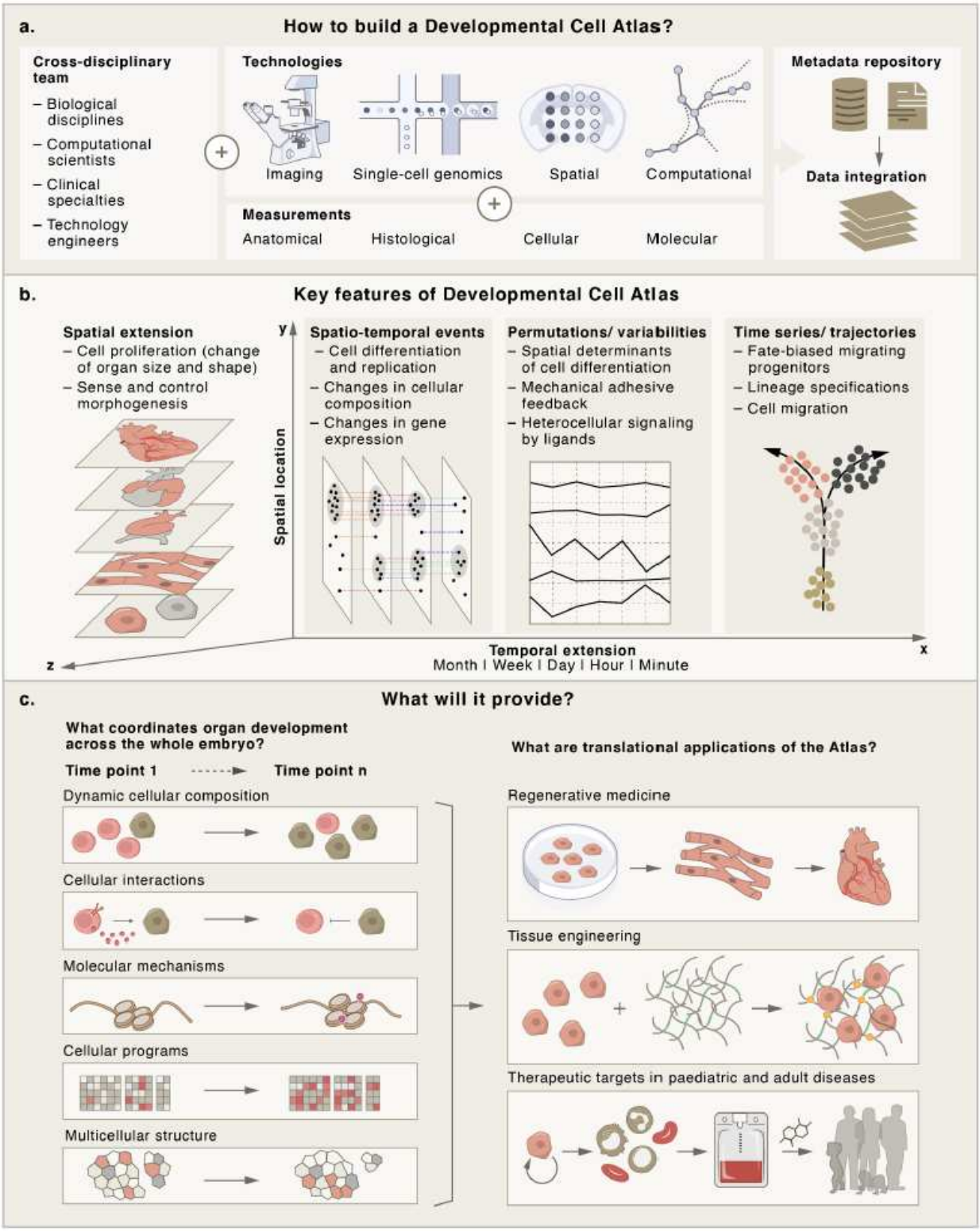

Figure 2

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.

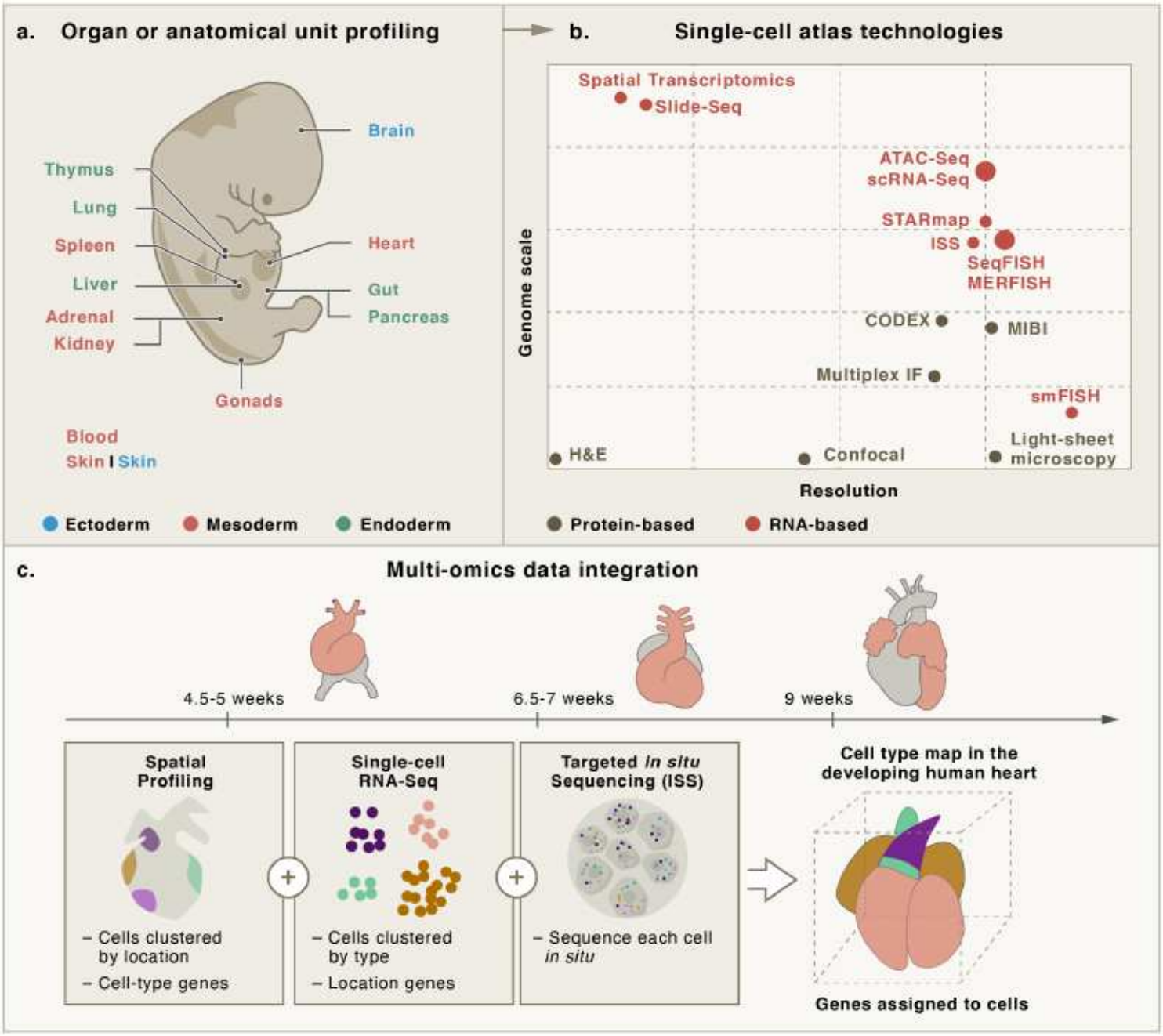

Figure 3

Multi-omics profiling and data integration a. Organ or anatomical unit profiling of a prenatal embryo derived from the three 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 embryo.

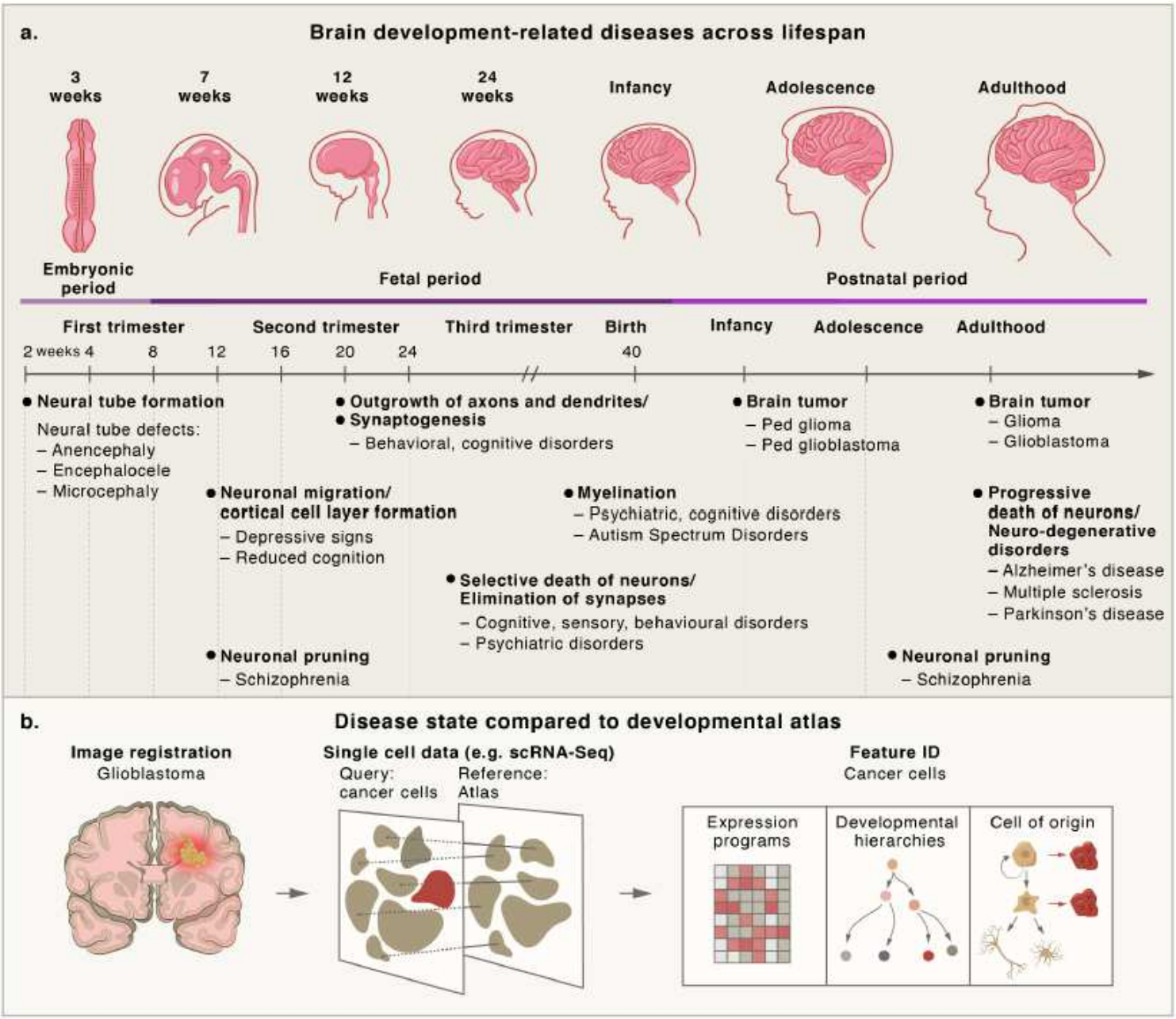

Figure 4

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.

Supplementary Files

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- Table.pdf