Single-cell and spatial transcriptomics during human organogenesis

The molecular and cellular events that occur during the onset of human organogenesis remain mysterious. We used single-cell and spatial transcriptomics to provide a global view of human embryonic cell-type specification, shedding light on developmental processes such as axial patterning, stage transition, and differences between human and mouse embryonic development.

This is a summary of:
Xu, Y. et al. A single-cell transcriptome atlas profiles early organogenesis in human embryos. *Nat. Cell Biol*. https://doi.org/10.1038/s41556-023-01108-w (2023).

Published online: 22 March 2023

The project

The vast cellular diversity of the human body traces its origin to embryonic organogenesis. During this critical developmental period, the precursors for most human cell types have already been established; defects in these cells can lead to miscarriage or birth defects. Despite its clinical importance, however, a pan-embryo cell atlas is not available. Moreover, most studies of early development have been conducted in model systems, such as mice and zebrafish, and the degree to which these studies reflect human embryogenesis remains controversial. With this in mind, we set out to provide a comprehensive single-cell transcriptome atlas of human embryos during organogenesis, which would provide a comprehensive reference for studying human embryonic development.

The observation

We obtained seven morphologically normal human embryos at 4–6 weeks of development — a stage at which major organs and tissue types begin to differentiate — and performed single-cell RNA sequencing (scRNA-seq) on dissected embryonic body sections (Fig. 1a). In total, we obtained 185,140 high-quality cells and defined 313 clusters from 18 developmental systems. With curation of ontology and marker genes from 157 references, we generated 177 unique terms in the annotation of cell types, including small anatomical structures, such as major embryonic signalling centres and sensory placodes, as well as migratory cell types, such as the second heart field. To elucidate spatial organization of cell types, we performed spatial transcriptomics on two sagittal sections from a Carnegie stage 13 human embryo (Fig. 1b). Integration of scRNA-seq and spatial transcriptomics revealed that the two datasets were highly coherent. Showing how these data can be used to explore previously under-characterized developmental systems, we focused on the head mesoderm[1], and detected five previously unappreciated cell types that have distinct spatial distribution. Thus, we provided a compendium of cell types with spatial information, which is particularly valuable for developmental systems for which there is limited understanding.

We further demonstrated that pan-embryo single-cell transcriptome information can elucidate important developmental processes, such as embryonic pattern formation. Through case studies of the limb bud and neural tube, we reconstructed the spatial axes and shed light on the heterogeneity of lineage differentiation and human-versus-mouse differences in developmental regulation. Notably, the prediction from single-cell data was independently validated by spatial transcriptome data for ventral expansion of *MSX2* expression in the human neural tube.

Finally, the systematic nature of the dataset provides an opportunity for gaining global views of developmental regulation that are not obvious from individual organ studies. Combining our human data with expression data in zebrafish, frog and mouse, we showed that vertebrate embryogenesis consists of four conserved developmental stages with sharp temporal boundaries. We discovered that *LIN28A*, a heterochronic gene in *Caenorhabditis elegans*, shows conserved and systemic up- and down-regulation of expression across cell types that corresponds to such developmental stage transitions, and may contribute to the systemic temporal regulation of vertebrate embryogenesis. Thus, our study puts forward a provocative but novel concept of staging for vertebrate embryogenesis.

Future directions

Human embryo studies are often limited due to ethical or practical limitations. As clinical specimens for stages prior to 4 weeks are extremely rare, our dataset provides a glimpse of the earliest stage of in vivo human embryonic development.

In terms of a complete and contiguous lineage trajectory, more embryonic stages need to be analysed. As shown in our effort to integrate our work with later-stage foetal organ data[2], data from 6 weeks to 10 weeks are crucial for trajectory tracing. Novel organoid and gastruloid models may provide critical insights into the first 4 weeks. For these, our dataset would serve as an in vivo benchmark for evaluating how well such models mimic true human development.

Accumulation of single-cell studies and data integration are key for consortium efforts such as the Human Cell Atlas[3]. Beyond transcriptome studies, multi-omics data such as chromatin accessibility, histone modification and DNA methylation would add invaluable insight into embryonic cellular differentiation.

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EXPERT OPINION

"The authors present the production and analysis of single-cell transcriptomic data from 4- to 6-week human embryos. Such data are very useful to the field and allow the posing of new questions about human development. The authors combine these data with other datasets in order to make some observations about spatial patterning, temporal regulation and later human development. I find the work a very compelling resource and of great benefit to the community." **An anonymous reviewer.**

FIGURE

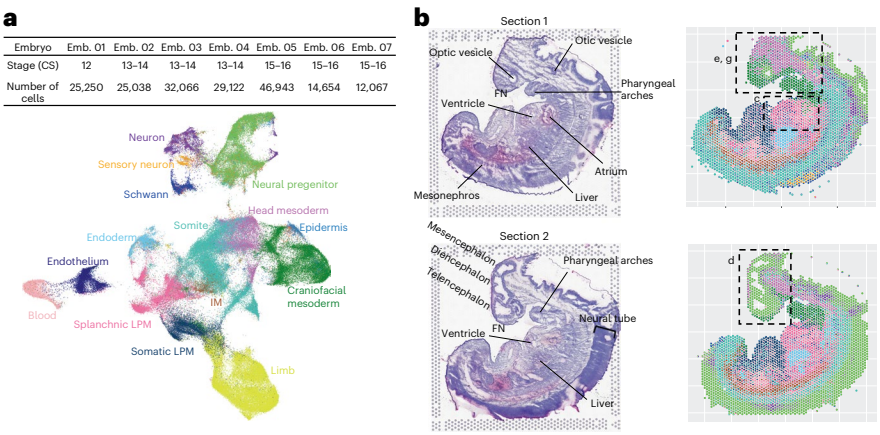

Fig. 1 | scRNA-seq and spatial transcriptome analysis of early human embryos. a, Top, Carnegie stage (CS) and number of high-quality cells in each embryo (Emb.) for scRNA-seq. Below, uniform manifold approximation and projection of high-quality cells; colours indicate developmental systems. IM, intermediate mesoderm; LPM, lateral plate mesoderm. **b**, Left, two sagittal sections of a Carnegie stage 13 human embryo in the spatial transcriptome (10x Visium). Recognizable structures on haematoxylin and eosin staining are annotated. FN, frontonasal mesenchyme. Right, the proportion of developmental systems (colours as in **a**) in each spot by deconvolution with scRNA-seq data.

BEHIND THE PAPER

The diversity of cell types increases substantially during organogenesis. The biggest challenge in this study was certainly the annotation of cell types, as there is not a comprehensive and curated list of cell types, marker genes and lineage relationships at this developmental stage. We (authors Y.X., T. Zhang, Z. Bao and W.S.) decided to collect and curate papers from the literature that studied human or mouse embryogenesis at the corresponding stages. For each developmental system, T. Zhang performed the initial screen and collected dozens of papers. Then, the four of us held online meetings twice a week to assemble a framework for annotation from those papers. We ended up with 157 papers and 234 marker genes. The process helped us keep our sanity during the early phase of the COVID-19 pandemic. We hope the community will join forces to enrich and improve the collection. **Y.X. & W.S.**

REFERENCES

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   This paper reports the distribution of somitomeres in head mesoderm in the mouse embryo.
2. Cao, J. et al. A human cell atlas of fetal gene expression. *Science* **370**, eaba7721 (2020).
   This paper reports single-cell transcriptomics of multiple human organs from 10 weeks to 26 weeks.
3. Regev, A. et al. The Human Cell Atlas White Paper. Preprint at https://doi.org/10.48550/arXiv.1810.05192 (2018).
   This paper reports the vision of the Human Cell Atlas project.

FROM THE EDITOR

"Early embryogenesis, especially in humans, is very much underexplored and has only recently started to reveal its secrets, following the advent of new technologies. This work by Xu et al. stood out to me because it characterizes a developmental window that spans early organogenesis in human embryos. The dataset could contribute to the understanding of both normal and disease-linked developmental events that have been so far a mystery."
Stylianos Lefkopoulos, Associate Editor, *Nature Cell Biology*.