Research Advance

Comments on ‘Molecular architecture of lineage allocation and tissue organization in early mouse embryo’

Guizhong Cui1, Naihe Jing1,2,4,*, and Guangdun Peng1,3,4, *

1 Guangzhou Regenerative Medicine and Health Guangdong Laboratory (GRMH-GDL), Guangzhou, China
2 State Key Laboratory of Cell Biology, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai, China
3 Key Laboratory of Regenerative Biology and Guangdong Provincial Key Laboratory of Stem Cells and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
4 Institute for Stem Cells and Regeneration, Chinese Academy of Sciences, Beijing, China

*Correspondence to: Naihe Jing, E-mail: njing@sibcb.ac.cn; Guangdun Peng, E-mail: peng_guangdun@gibh.ac.cn

Single-cell RNA-seq, with its capability to align cells of continuously changed status by pseudo-time reconstruction, has greatly revolutionized the understanding of cell fate transition during embryo development (Shapiro et al., 2013; Hoppe et al., 2014). While there is still a lack of single-cell spatial analysis, with the spatial variance contributing to the cell alignment, pseudo-space analysis might be conducted and the cell organization could be inferred as well (Cheng et al., 2019; Nowotschin et al., 2019). However, rather than revealing spatial or developmental trajectory by computational reconstruction, transcriptomic analysis of real time and space provides an authentic benchmark for dissecting the cell organization, molecular architecture, and lineage allocation. The ability to discern spatial gene expression differences in complex biological systems is critical to our understanding of developmental biology and the progression of disease.

In the recent publication entitled ‘Molecular architecture of lineage allocation and tissue organization in early mouse embryo’ (Peng et al., 2019), we performed a systematic survey of spatial architecture of post-implantation mouse embryos spanning E5.5 to E7.5 stages. In contrast to conventional single-cell RNA-seq of early mouse embryos at the post-implantation stages, which is quite a few now (Pijuan-Sala et al., 2019; Nowotschin et al., 2019), the native location of cells and the relationship between cells were retained, thus providing unique attributes to probe the dynamic molecular structure of progenitor cells in the embryo.

This is a data-heavy work, considering that so many pieces of laser microdissected embryonic tissues were sequenced. Although the 2D display and identification of spatial domains were basically following the previous endeavors (Peng et al., 2016; Han et al., 2018), the tissue lineage and connectivity of cell populations in time and space demand unique analytic methodologies that differ greatly with single-cell trajectory protocol. The relatively sparse data coverage does not fit a continuous change model for pseudo-time or pseudo-space reconstruction. Besides, various degrees of batch effects were introduced during integration of a huge number of data sets.

We employed a pipeline called SCENIC that includes gene regulation network as regulon units (Aibar et al., 2017). It can migrate batch effect efficiently and identify stable biological significance (Suo et al., 2018). Combined with published data set from pre-implantation mouse embryos, our analysis, in this way, revealed the transition and segregation of cell lineages and established the path for progenitor cells evolving in real time and space.

The strides for the spatiotemporal transcriptomic analysis, besides being valuable resource for digital whole mount in situ hybridization of > 20000 transcribed coding and noncoding genes, uncovered two unappreciated tissue relationships not known by conventional fate mapping techniques. One is the surprisingly high contribution of extraembryonic endoderm to embryonic endoderm lineage. It is intriguing that the extraembryonic endoderm profoundly shares gene signatures with embryonic endoderm (Moerkamp et al., 2013). There even have been lineage tracing evidences showing that visceral endodermal deccents were found in the differentiated gut (Kwon et al., 2008; Chan et al., 2019).

© The Author(s) (2019). Published by Oxford University Press on behalf of Journal of Molecular Cell Biology, IBCB, SIBS, CAS.
Our data, from the viewpoints of transcriptomic profiles and regulatory mechanisms (by SCENIC), substantiated the visceral endodermal origination of definitive endoderm. Furthermore, we collected single cells from the endoderm tissue layers and identified three single-cell clusters. One cell cluster contains primitive streak marker T/Mix1, which might be potential mesendoderm progenitors, or mesoderm and endoderm cells migrating together. Importantly, the other two single-cell clusters are predominately enriched for visceral endodermal markers, indicating a convergence of two endoderm origins. However, whether these two sources of endodermal cells have different developmental potentials await further experimental verification. The other surprising finding is the close relationship between posterior ectoderm and posterior mesoderm at E7.0, which may explain the presence of progenitors for spinal cord and somitic mesoderm (Henrique et al., 2015). Our unpublished data on single-cell mapping also indicated that Sox2/T double-positive cells are residing in the corresponding locations suggested by previous clonal analysis.

The spatiotemporal transcriptome reveals new signaling players in gastrulation mouse embryos. Hippo/Yap signaling pathway, an important determinant during the inner cell mass and trophoderm segregation, showed activation in the visceral endoderm tissues. By inhibiting Hippo/Yap ex vivo, we showed that the transition of extraembryonic and embryonic endoderm lineages is likely dependent on tissue-specific activity of Hippo/Yap signaling. We reasoned that a Hippo/Yap (activated in the visceral endoderm tissues) and Nodal (expressed in the epiblast tissues) signaling axis may contribute to the endoderm development at early epiblast unto late lineage commitment, respectively.

However, the drawbacks of lineage reconstruction on the basis of spatial transcriptome or single-cell RNA-seq are limitations resulted from the inherent gene expression approach. Although unprecedented details were obtained, the transcriptomic data are still representations of static snapshots of cells or cell population, which is not akin to genetic lineage tracing and live imaging. Moreover, many of the data are sparse and have very high dimensionalities, making the prediction of tissue lineage very difficult (Kretzschmar and Watt, 2012; Kester and van Oudenaarden, 2018). A further refinement of computational lineage reconstruction and a combination with genetic lineage tracing would be essential for scrutinizing many paradigms of tissue lineages established by conventional low-resolution approaches in early embryo development and stem cell biology (Figure 1).

Figure 1 Remaining challenges for lineage reconstruction by spatiotemporal transcriptome analysis.

[The work was supported in part by the National Key Basic Research and Development Program of China (2018YFA0108000, 2018YFA0800100, 2017YFA0102700, 2015CB964500, and 2014CB964804 to N.J.; 2018YFA0107201 to G.P.), the ‘Strategic Priority Research Program’ of the Chinese Academy of Sciences (XDA16020501 to N.J.; XDA16020404 to G.P.), the National Natural Science Foundation of China (31871456 to G.P.; 31661143042, 91519314, 31630043, 31571513, and 31430058 to N.J.), Shanghai Natural Science Foundation (18ZR1446200), Science and Technology Planning Project of Guangdong Province (2017B030314056), and Frontier Research Program of Guangzhou Regenerative Medicine and Health Guangdong Laboratory (2018GZR110105013).]

References
Albar, S., González-Blas, C.B., Moerman, T., et al. (2017). SCENIC: single-cell regulatory network inference and clustering. Nat. Methods 14, 1083–1086.
Chan, M.M., Smith, Z.D., Grosswendt, S., et al. (2019). Molecular recording of mammalian embryogenesis. Nature 570, 77–82.
Cheng, S., Pei, Y., He, L., et al. (2019). Single-cell RNA-seq reveals cellular heterogeneity of pluripotency transition and X chromosome dynamics during early mouse development. Cell Rep. 26, 2593–2607.e3.
Han, X., Luo, S., Peng, G., et al. (2018). Mouse knockout models reveal largely dispensable but context-dependent functions of IncRNAs during development. J. Mol. Cell Biol. 10, 175–178.
Henrique, D., Abranches, E., Verrier, L., et al. (2015). Neuronesdernal progenitors and the making of the spinal cord. Development 142, 2864–2875.
Hoppe, P.S., Coutu, D.L., and Schroeder, T. (2014). Single-cell technologies sharpen up mammalian stem cell research. Nat. Cell Biol. 16, 919–927.
Kester, L., and van Oudenaarden, A. (2018). Single-cell transcriptomics meets lineage tracing. Cell Stem Cell 23, 166–179.
Kretzschmar, K., and Watt, F.M. (2012). Lineage tracing. Cell 148, 33–45.
Kwon, G.S., Viotti, M., and Hadjantonakis, A.K. (2008). The endoderm of the mouse embryo arises by dynamic widespread intercalation of embryonic and extraembryonic lineages. Dev. Cell 15, 509–520.
Moerkamp, A.T., Paca, A., Goumans, M.J., et al. (2013). Extraembryonic endoderm cells as a model of endoderm development. Dev. Growth Differ. 55, 301–308.
Nowotschin, S., Setty, M., Kuo, Y.Y., et al. (2019). The emergent landscape of the mouse gut endoderm at single-cell resolution. Nature 569, 361–367.
Peng, G., Suo, S., Chen, J., et al. (2016). Spatial transcriptome for the molecular annotation of lineage fates and cell identity in mid-gastula mouse embryo. Dev. Cell 36, 681–697.
Peng, G., Suo, S., Cui, G., et al. (2019). Molecular architecture of lineage allocation and tissue organization in early mouse embryo. Nature 572, 528–532.
Pijuan-Sala, B., Griffiths, J.A., Guibentif, C., et al. (2019). A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566, 490–495.
Shapiro, E., Biezuner, T., and Linnarsson, S., (2013). Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. 14, 618–630.
Peng, G., Suo, S., Cui, G., et al. (2019). Molecular architecture of lineage allocation and tissue organization in early mouse embryo. Nature 572, 528–532.
Pijuan-Sala, B., Griffiths, J.A., Guibentif, C., et al. (2019). A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566, 490–495.
Shapiro, E., Biezuner, T., and Linnarsson, S., (2013). Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. 14, 618–630.
Peng, G., Suo, S., Cui, G., et al. (2019). Molecular architecture of lineage allocation and tissue organization in early mouse embryo. Nature 572, 528–532.
Pijuan-Sala, B., Griffiths, J.A., Guibentif, C., et al. (2019). A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566, 490–495.
Shapiro, E., Biezuner, T., and Linnarsson, S., (2013). Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. 14, 618–630.
Suo, S., Zhu, Q., Sadatpour, A., et al. (2018). Revealing the critical regulators of cell identity in the mouse cell atlas. Cell Rep. 25, 1436–1445.e3.