

Cell Lineage Specification at Single Cell Resolution

Yang J^{*} and Liu P

Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1HH, UK

^{*}Corresponding author: Yang J, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK, Tel: 44-0-1223834244-8786; E-mail: jy4@sanger.ac.uk

Rec date: August 22, 2017; Acc date: August 24, 2017; Pub date: August 26, 2017

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Abstract

Single cell technology has been widely used in developmental and stem cell biology. In mouse, single cell transcriptome has revealed the cell lineage specification in pre-implantation and post-implantation stage embryo. Now Mohammed et al. investigated the dynamic cell fate commitment during the transition from peri-implantation to early post-implantation stage with single cell RNA sequencing. Except confirmation of some previous findings, the time window and cell sub clusters of cell specification is more precisely determined, along with possible new mechanism for X re-activation and inactivation in female embryo and for exit from pluripotency and lineage commitment. These data will not only fill the missing link in mouse embryo development, but provide insights into embryo development of other mammalian species including human.

Keywords: Single cell; Embryo; Lineage specification; Development

Introduction

How a zygote generates a multicellular organism is one of the fundamental questions in developmental and stem cell biology. Take mouse embryo development as an example. Until 8-cell stage, each blastomere is totipotency, i.e., it can generate the first three lineages, trophoctoderm (TE), epiblast (EPI) and primitive endoderm (PE); though recent reports showed a single 4 cell blastomere's contribution to TE/ICM is biased and single cell assay also revealed heterogeneity in gene expression. At morula stage (16-32 cells), the first two lineages, TE and ICM are segregated, then at blastocyst stage, ICM specified into EPI and PE. After implantation, TE contributes to trophoblasts in placenta; EPI produces all fetal cells including germ cells and PE mostly forms extra-embryonic yolk sac [1].

Cell Lineage Specification

In order to trace the cell specification during development, various methods and techniques have been developed and applied, including cell labelling, transplantation of cells and tissues, introduction of genetic markers by transfection or viral transduction and cell marking by genetic recombination etc. All these methods have pros and cons though [2]. More recently, with the fast advance of single cell technology, the fate of cells can be monitored more precisely and in more details [3]. Early this year, Posfai et al. using single cell RNA sequencing found drastically increased diversity among cells between later 16 cells stage and early 32 cells stage, suggesting the emergence of TE and ICM lineages at these stages [4]. Before, Peng et al. constructed a spatial transcriptome of late mid-streak embryo (around embryo day (E)7.0) using high resolution RNA sequencing (20 cells from four positions in each section) and correlated with regionalization of cell fates in the embryo. From these dataset, the authors created zip code mapping. To test the utility of the map, they sequenced 70 single cells, after inputting the data, the position of single cell in embryo was mapped. It turned out that 66 of 70 single cells can be mapped back to the original half-epiblast [5].

There is a gap between these two reports which is the transition of embryo from peri-implantation to post-implantation. During these stages, ICM segregates into EPI and PE, EPI subsequently undergo expansion to form primitive streak to initiate gastrulation. This is also critical time window for stem cell biology as naïve embryonic stem cells (ESCs) can be derived from ICM in blastocyst, whereas primed epiblast stem cells (EpiSCs) were derived from post-implantation EPI and the transcriptional and epigenetic changes during the exit from naïve pluripotency is still not clear [6]. To open the black box, now Mohammed et al. performed single cell RNA sequencing of ICM of E3.5, EPI and PE at E4.5, E5.5 and E6.5. E3.5 cells expressed high level of pluripotency genes; at E4.5, EPI and PE was clearly separated; at E5.5, naïve pluripotency gene *Nanog* was down-regulated accompanied by increased primed pluripotency maker *Pou3f1*; at E6.5, the embryo cluster into four groups, visceral endoderm (from PE), a primitive streak population and two sub clusters of non-committed epiblast cells [7].

In female mouse embryo, between E3.5 and E4.5, the paternal X chromosome is reactivated, then from E5.5 on, one X chromosome is randomly inactivated [8]. Consistent with the *in vivo* phenomenon, female naïve ESCs also have two activated X chromosomes whereas one X chromosome is inactivated in female EpiSCs [6]. The new single cell transcriptome data confirmed the previous observation, moreover, they found strong association between X reactivation and *Pou5f1* and one of its interacting partner *Zfhx3* at E3.5; while increased expression of *Dnmt3a* and *Zfp57* is associated with the following X inactivation, which will provide further insights into the mechanism of X reactivation and inactivation [7].

Prior to the segregation of EPI and PE at E4.5, specific epiblast and endoderm genes are co-expressed at E3.5. The authors' data clearly showed genes enriched for EPI and PE respectively at E4.5; at E3.5, though subsets of EPI and PE genes were expressed, no lineage subgroups were observed. Besides identifying genes such as *Fgf4* and *Fgfr2* known to derive EPI and PE specification, they also found *Pdgfra*, *Top2b*, *Sox17*, *Gata4* and *Pdk2* was associated with PE fate, and *Morc1*, *Nanog*, *Dppa5* and *Pdpn* was associated with EPI fate [7].

From E5.5 to E6.5, the cells begin to exit pluripotency and undergo lineage commitment. The data revealed that at E5.5, though some genes associated with anterior-posterior polarity and the primitive streak, no apparent substructure or expression of primitive streak markers were expressed, whereas at E6.5, four subgroups were identified as mentioned above, in EPI, the expression of *Otx2*, a gene associated with exit from pluripotency increased, along with several polycomb genes, which suggests the importance of polycomb complex in establishing transcriptional control in the non-committed epiblast cells [7].

Discussion and Conclusion

Finally, they investigated the transcriptional noise at different embryo stages, which showed high noise at E3.5 unspecified cells, then gradually reduced and at E6.5, the primitive streak has much lower noise than the uncommitted epiblast. These data suggest that there is possibility of increase transcriptional noise before lineage commitment as also observed in other systems [7,9].

In summary, this study provides us more information about the dynamic changes during the transition from peri-implantation to early post-implantation stages, which mark the lineage segregation, exit from pluripotency and differentiation. However, the commitment of TE is missing in this report. Considering trophoblast stem cells can be derived from either E3.5 blastocyst or E6.5 extra-embryonic ectoderm [10]. It'll be interesting to investigate the fate of TE during this transition stage at single cell level.

The new study may also shed light on the embryo development of human and other mammalian species. Several reports investigated transcriptome of human pre-implantation embryos by single cell sequencing, from which not only a number of genes with conserved expression as in mouse was observed, but also significant difference between human and mouse was identified [11,12]. Moreover, X reactivation and inactivation in female human blastocyst is different as well [13]. Because of the ethical issues, it's almost impossible to study the early human post-implantation embryos. However, recently developed *in vitro* culture systems with which human blastocyst can self-organize into post-implantation embryo like structure may facilitate the study of human post-implantation embryo development [14,15].

In all, the advanced single cell technology will provide a powerful tool for developmental and stem cell scientists to map the cell fates

from a zygote to the whole organism which can be translated into clinical application.

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