Decoding Extra-embryonic Hematopoiesis of Early Embryogenesis Using Hex-embryoids

Ziyu Yu[1,2,4], Kamyar Keshavarz F.[2,4], Joshua Hislop[2], Rayna Schoenberger[2], Hao Chen[3], Samira Kiani[2], Ziv Bar-Joseph[3], Mo R. Ebrahimkhani[2]

[1]Tsinghua University, school of medicine

[2]University of Pittsburgh & Pittsburgh Liver Research Center

3Computational Biology Department, School of Computer Science, Carnegie Mellon

4These authors contribute equally

Understanding early human hematopoiesis is crucial for advancing treatments for immune cell-related diseases and for generating functional blood progenitors for transplantation. Immediately after implantation, extra-embryonic tissues co-develop with the embryo, supporting nutrient exchange, generating the first germ cells, and, most importantly, supplying the earliest blood cells in the yolk sac, which persist throughout life. However, a comprehensive study of yolk sac hematopoiesis has been hindered by both ethical constraints and technical challenges. Recently, human iPSC-derived embryoid models have been developed to address this gap in our understanding. The human yolk sac originates from GATA6-expressing hypoblasts, which are specified at the late blastocyst stage. Inspired by this natural process, our laboratory has developed a human embryoid model that incorporates an extra-embryonic niche and recapitulates yolk sac hematopoiesis, termed "*heX-embryoid*."

By generating a heterogenous cell line with different expression levels of GATA6, and combining them with wild-type iPSC, the cells go through self-organization and 3D assembling, resulting in the formation of the bilaminar disc and gastrulation-like events in the epiblast. Notably, we observe multilineage morphogenesis of the extra-embryonic yolk sac, with blood island-like structures containing endothelial vessels interspersed between endodermal and mesodermal cells, where CD43+ blood cells emerge. We confirmed its yolk sac identity through single cell sequencing, while the tissue lacks HOXA9 gene but expresses embryonic genes e.g., LIN28A shown in early waves of hematopoiesis. We also detect the specification of cells expressing markers for lymphoid (CD7, IL7R), erythroid (Hb), megakaryocytes (CD42b), leukocyte (CD45) and myeloid/macrophage (CD33, CXCR1) lineages, with a sub-cluster resembling primitive macrophages with similarity to microglia and Kupffer cells. Furthermore, spatiotemporal analysis reveals distinct hematopoietic foci: CD7+ lymphoid lineage generation depends on the embryonic body, where they emerge in the vasculature closely connected with the wild-type cluster, while myeloid (CD45), erythroid (Hb), and megakaryocyte (CD42b) lineages develop independently of the embryonic body and could appear randomly on the culture dish. Additionally, transplantation of iPSC-derived yolk sac tissue into immuno-compromised mice led to the appearance of human CD68+ macrophages in various tissues, including skin, spleen, liver, and lung, suggesting that progenitors from the yolk sac-like niche can implant to multiple macrophage niches. In conclusion, heX-embryoids provide a powerful platform for decoding early hematopoiesis in humans and enable the generation of hard-to-access cell types. This model holds promise for future applications in transplantation and cell-based therapies.