

Single cell approaches

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A new lymphoid-primed progenitor marked by Dach1 downregulation identified with **single cell** multi-omics.

Amann-Zalcenstein D, Tian L, Schreuder J, Tomei S, Lin DS, Fairfax KA, Bolden JE, McKenzie MD, Jarratt A, Hilton A, Jackson JT, Di Rago L, McCormack MP, de Graaf CA, Stonehouse O, Taoudi S, Alexander WS, Nutt SL, Ritchie ME, Ng AP, Naik SH.

Nat Immunol. 2020 Oct 19. doi: 10.1038/s41590-020-0799-x. Online ahead of print.

Tracing the first hematopoietic stem **cell** generation in human embryo by **single-cell** RNA sequencing.

Zeng Y, He J, Bai Z, Li Z, Gong Y, Liu C, Ni Y, Du J, Ma C, Bian L, Lan Y, Liu B. Cell Res. 2019 Nov;29(11):881-894. doi: 10.1038/s41422-019-0228-6. Epub 2019 Sep 9.

Single-Cell Analyses Reveal Megakaryocyte-Biased Hematopoiesis in

Myelofibrosis and Identify Mutant Clone-Specific Targets. Psaila B, Wang G, Rodriguez-Meira A, Li R, Heuston EF, Murphy L, Yee D, Hitchcock IS, Sousos N, O'Sullivan J, Anderson S, Senis YA, Weinberg OK, Calicchio ML: NIH Intramural Sequencing Center, Iskander D, Royston D, Milojkovic D, Roberts I, Bodine DM, Thongjuea S, Mead AJ. Mol Cell. 2020 May 7;78(3):477-492.e8. doi: 10.1016/j.molcel.2020.04.008.

Combinatorial **Single-Cell** Analyses of Granulocyte-Monocyte Progenitor Heterogeneity Reveals an Early Uni-potent Neutrophil Progenitor. Kwok I, Becht E, Xia Y, Ng M, Teh YC, Tan L, Evrard M, Li JLY, Tran HTN, Tan Y, Liu D, Mishra A, Liong KH, Leong K, Zhang Y, Olsson A, Mantri CK, Shyamsunder P, Liu Z, Piot C, Dutertre CA, Cheng H, Bari S, Ang N, Biswas SK, Koeffler HP, Tey HL, Larbi A, Su IH, Lee B, St John A, Chan JKY, Hwang WYK, Chen J, Salomonis N, Chong SZ, Grimes HL, Liu B, Hidalgo A, Newell EW, Cheng T, Ginhoux F, Ng LG. Immunity. 2020 Aug 18:53(2):303-318.e5. doi: 10.1016/j.immuni.2020.06.005. Epub 2020 Jun 23.

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Single-cell lineage tracing approaches in hematology research: technical

considerations.

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Exp Hematol. 2020 Sep;89:26-36. doi: 10.1016/j.exphem.2020.07.007. Epub 2020 Jul 28.

Resolving Fates and **Single-Cell** Transcriptomes of Hematopoietic Stem **Cell** Clones by PolyloxExpress Barcoding.

Pei W, Shang F, Wang X, Fanti AK, Greco A, Busch K, Klapproth K, Zhang Q, Quedenau C, Sauer S, Feyerabend TB, Höfer T, Rodewald HR.

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- Key to understanding organs/tissues is finding active genes
- Organs/tissues consist of functionally different cell types
- Characterisation of single cells has emerged as key driver to unravel tissue biology





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Haematopoiesis; Paradigm for hierarchically organised and heterogeneous tissue





- Blood consists of >10 mature cell types
- The are all produced by Hematopoietic Stem Cells (HSC)
- The differentiation pathways remain under controversy



Classic haematopoietic hierarchy



Progenitor populations phenotypically defined:

CMP: Common Myeloid Progenitor GMP: Granulocyte and Monocyte Progenitor MEP: Megakaryocyte and Erythroid Progenitor



Revised haematopoietic hierarchy



- LMPP: Lymphoid primed multipotent progenitor
- Phenotypic CMP consist of distinct subpopulations



Adolfsson-Jacobsen, Cell 2005 Pronk-Bryder, Cell Stem Cell 2007 Perié-Schumacher, Cell 2015 Paul-Amit, Cell 2015

Decipher hierarchy by single cell gene expression



- Are the correct cells sequenced?
- Is there biological proof of cell fate?
- Sub-populations need to be identifiable

PreGM population consist of two subpopulations



Gata1 expression marks two preGM subpopulations



- Transgenic mouse model; Gata1 expressing cells are EGFP⁺
- FACS to prospectively isolate Gata1⁻ and Gata1⁺ cells
- Culture cells to test biological potential

preGM subpopulations have different lineage potential



preGM Gata1+



preGM Gata1-

Gata1⁺ preGM produce:

- mast cells and eosinophils
- •

Gata1⁻ preGM produce:

• neutrophils and monocytes

•



Erythrocyte/Megakaryocyte potential is restricted to *Gata1*⁺ progenitors Lymphoid potential is restricted to *Gata1*⁻ progenitors



Myeloid cells are produced via separate pathways



Importance of establishing the haematopoietic hierarchy



• Being able to study the molecular mechanisms behind lineage decisions

Importance of establishing the haematopoietic hierarchy



- Being able to study the molecular mechanisms behind lineage decisions
- Finding the Leukemic Initiating Cell (LIC) in cancers

Other techniques

SmartSeq:	Relative low amount of cell, high sequencing depth
10xChromium:	High number of cell, lower sequencing depth
CITE-seq:	Cellular Indexing of Transcriptomes and Epitopes by sequencing:
	Barcode brought to cells via cell surface markers
ATAC-seq:	Assay for Transposase-Accessible Chromatin using sequencing
	Detecting open chromatin as opposed to expressed genes





Summary

- Tissues consist of functionally distinct cell types
- Single cell techniques unravel heterogeneity of tissues
- Gene expression is an indication, but not proof, for the potential of a cell
- Prospective identification and isolation of cell types is important for studying the cellular hierarchy
- Single cell technologies are rapidly improving; choose the best technique for your biological question