Hepatology Snapshot:

A single cell gene expression atlas of 28 human livers

JOURNAL
OF HEPATOLOGY

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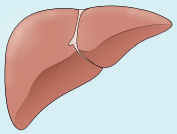
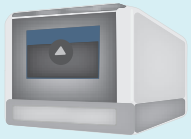
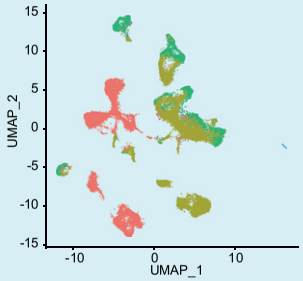
28 Human healthy livers



Single cell RNA-sequencing (scRNA-Seq)

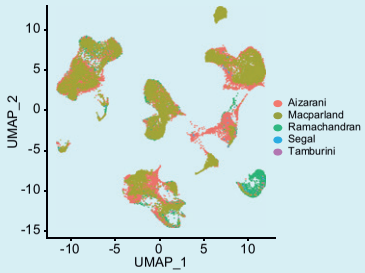


Unintegrated data from 5 studies



Seurat was used to integrate data

Integrated data

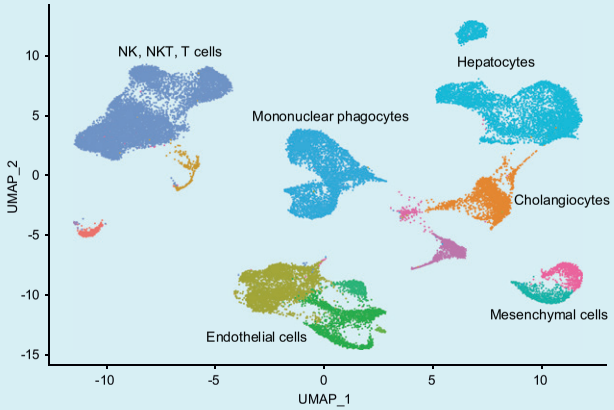


Integration of single cell expression data is a new computational approach developed to deal with the rapidly expanding availability of scRNA-seq data. Seurat was used to succesfully integrate these 5 datasets, using a marker gene strategy to integrate across independent studies.

Study	Number of healthy livers	Gender (M:F)	Age (yr-old)	Method of liver collection	Sequencing method	Enriched for	Final # healthy cells
Aizarani *et al. Nature* 2019	9	n/a	n/a	Liver resection without history of chronic liver disease	mCel-Seq2	None	11,868
Macparland *et al. Nature Communications*, 2018	5	4M:1F	21-65	Caudate lobe of livers from neurologically deceased donors	10x Chromium	None	12,476
Ramachandran *et al. Nature 2019*	5	4M:1F	57.4 ± 5.8	Intraoperative liver resection during surgery for colorectal metastases	10x Chromium	CD45- and CD45+ cells	11,570
Segal *et al. Nature Communications*, 2019	3	n/a	n/a	Unknown	SmartSeq2	Biliary cells and hepatocytes	94
Tamburini *et al. Frontiers in Immunology, 2019*	6	5M:1F	37-58	Non-diseased livers from deceased individuals from *Lonza Biosciences Solutions*	10x Chromium	Endothelial cells	180

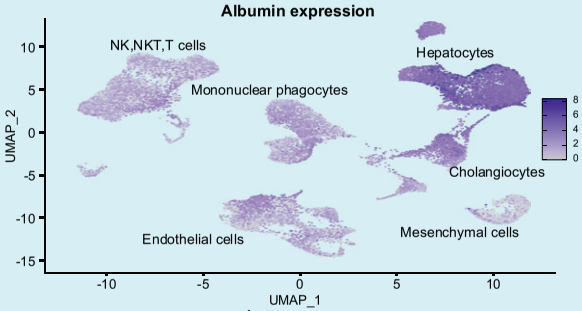
The integration of scRNA-seq data from 28 human livers, reported in 5 independent studies, expands the single cell liver atlas. It comprises liver single cell transcriptomics across various ages, sex, liver collection methods, and single cell sequencing technologies as outlined in the table above.

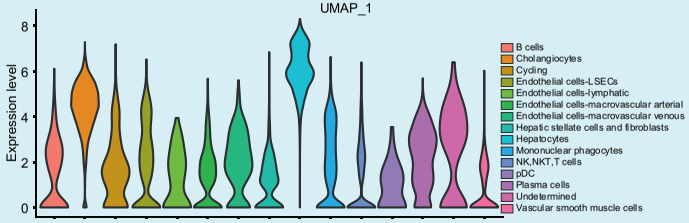
Cell types and subtypes in human livers



- B cells
- Cholangiocytes
- Cycling
- Endothelial cells-LSECs
- Endothelial cells-lymphatic
- Endothelial cells-macrovascular arterial
- Endothelial cells-macrovascular venous
- Hepatic stellate cells and fibroblasts
- Hepatocytes
- Mononuclear phagocytes
- NK, NKT, T cells
- pDC
- Plasma cells
- Undetermined
- Vascular smooth muscle cells

Interactive single cell human liver atlas browser





To facilitate exploration of this large dataset we have created an interactive browser that allows for the visualization and interrogation of gene expression (*e.g.* albumin) across multiple cell types. This tool enables rapid identification of liver expression when assessing gene candidates from both genomic analysis and other high throughput sequencing technologies.

http://liveratlas-vilarinholab.med.yale.edu

Keywords: transcriptomics, gene expression, cell heterogeneity
Received 26 November 2020; received in revised form 04 March 2021; accepted 10 March 2021.

Chronic liver disease (CLD) affects over 1 billion people worldwide[1] and non-invasive therapeutic options remain an unmet medical need. Thus, advances in uncovering the underlying molecular mechanism(s) of liver disease pathogenesis are essential to facilitate the discovery of novel therapeutic targets.

Next-generation sequencing technologies have revolutionized our understanding of human health and disease through its application to DNA and RNA sequencing. The latter comprises single-cell transcriptomics, which enables sequencing of thousands of cells in a single sample and analysis of gene expression of all cells individually. This creates an unbiased approach to assess cell identity and heterogeneity and to uncover rare cell populations otherwise obscured in bulk RNA-sequencing studies. Taking advantage of fully commercialized workflows, high-throughput single-cell RNA-sequencing (scRNA-seq) is increasingly being used to investigate the cell complexity and heterogeneity of human organs, including the liver. Whereas access to good quality fresh human healthy liver tissue has represented a major limitation in the field, in the last 2 years, 5 independent studies have analyzed human healthy livers at single cell resolution.[2-6] Thus, integration of these 5 scRNA-seq datasets can provide further insight into the transcriptomic architecture and stability of the human liver in physiological conditions, representing highly valuable, and previously unattainable, information for the liver research community worldwide. Herein, we summarize, integrate and analyze available human liver scRNA-seq data and provide an interactive open-access online cell browser for easy access to gene expression data across a variety of annotated parenchymal and non-parenchymal cells derived from 28 human healthy livers.

ScRNA-seq data was accessed from NCBI GEO and processed using Seurat v3.[7] All datasets were filtered to only include cells expressing 250-2,500 genes as well as mitochondrial expression below 30%. Once filtered and normalized, Seurat was used to batch correct between the independent sources. Detailed methods and R code can be found at http://liveratlas-vilarinholab.med.yale.edu/ and https://github.com/joeb-liver/Single_Cell_Liver_Atlas, respectively.

Liver samples were obtained from individuals of both sexes, a wide range of age groups (21–65-years-old) and with a variety of underlying medical conditions. These include (i) liver resections for colorectal cancer metastasis or cholangiocarcinoma without history of CLD[3]; (ii) liver tissue from neurologically deceased donors deemed acceptable for liver transplantation[2]; (iii) liver resection for solitary colorectal metastasis[6] and (iv) non-diseased liver tissue from deceased individuals which was not suitable for transplantation.[5] In aggregate, the merged dataset comprises 26 clusters of a total of 36,188 liver cells. Cell lineage was inferred from a combination of unbiased clustering and gene expression profiles. This human liver single cell atlas is composed by 6,895 hepatocytes, 2,357 cholangiocytes, 6,876 liver endothelial cells, 1,604 mesenchymal cells and 18,223 immune cells. Importantly, an R Shiny application was created for interactive visualization and can be accessed at http://liveratlas-vilarinholab.med.yale.edu. This new resource facilitates user-friendly access and interactive visualization of gene expression for each cell (sub)type, which encompasses a range of abundant to rare liver cell populations. Moreover, this new web tool also gives information on which and what proportion of cell (sub)types express a gene of interest.

Collectively, this snapshot compiles all available healthy human liver scRNA-seq data and provides a valuable online resource to compare gene expression across the diverse liver cell populations. This web portal was designed to deliver up-to-date single cell gene expression data accessible to any researcher worldwide working on liver-related biology independent of their bioinformatic training and skills. Hence, this new resource tool has the potential to accelerate basic, translational and clinical research in liver health and disease globally.

Financial support

Our laboratory is supported by the Doris Duke Charitable Foundation Grant #: 2019081 and the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health under Award Number K08 DK113109. J.B. is supported by the National Institute of General Medical Sciences of the National Institute of Health under Award Number 1T32GM136651-01.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

J.B. and S.V. conceptualized the study, analyzed the data and wrote the manuscript.

Acknowledgements

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.03.005.

References

Author names in bold designate shared co-first authorship

[1] Moon AM, Singal AG, Tapper EB. Contemporary epidemiology of chronic liver disease and cirrhosis. Clin Gastroenterol Hepatol 2020;18:2650–2666.
[2] MacParland SA, Liu JC, Ma XZ, Innes BT, Bartczak AM, Gage BK, et al. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun 2018;9:4383.
[3] Aizarani N, Saviano A, Sagar, Mailly L, Durand S, Herman JS, et al. A human liver cell atlas reveals heterogeneity and epithelial progenitors. Nature 2019;572:199–204.
[4] **Segal JM**, **Kent D**, Wesche DJ, Ng SS, Serra M, Oules B, et al. Single cell analysis of human foetal liver captures the transcriptional profile of hepatobiliary hybrid progenitors. Nat Commun 2019;10:3350.
[5] Tamburini BAJ, Finlon JM, Gillen AE, Kriss MS, Riemondy KA, Fu R, et al. Chronic liver disease in humans causes expansion and differentiation of liver lymphatic endothelial cells. Front Immunol 2019;10:1036.
[6] Ramachandran P, Dobie R, Wilson-Kanamori JR, Dora EF, Henderson BEP, Luu NT, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. Nature 2019;575:512–518.
[7] **Stuart T**, **Butler A**, Hoffman P, Hafemeister C, Papalexi E, Mauck 3rd WM, et al. Comprehensive integration of single-cell data. Cell 2019;177. 1888-1902 e1821.