Single cell RNA sequencing of the native human liver and its spatial resolution reveal new subpopulations of non-parenchymal cells.

Payen V.L.[1,2,4], Lavergne A.[3,4], Charloteaux B.[3], Alevra Sarika N.[1,2], Karim L.[3], Deckers M.[3], Najimi M.[1], Smets F.[1], Jazouli N.[1], Coppieters W.[3], Georges M.[3], Sokal E.M[1,4] and El Taghdouini A.[1,4*].

[1] Université catholique de Louvain (UCL) - Pediatric hepatology and cell therapy laboratory, Brussels, Belgium ; **[2]** Université catholique de Louvain (UCL) – Advanced drug delivery and biomaterial laboratory, Brussels, Belgium; **[3]** Université de Liège (ULg) – Medical genomics department (GIGA), Liège, Belgium. **[4]** Equal contributing authors. ***** Corresponding author.

Background: The liver's multitude of vital functions are tightly linked to its complex assembly of highly specialized parenchymal and non-parenchymal cells in collaborative sinusoidal units. In the present study we aimed at providing the first high-resolution transcriptomic map of the infant and adult human liver by single-cell RNA sequencing, as a reference to understanding liver physiology and disease. **Method:** ~80,000 liver cells obtained after collagenase digestion of two human livers were loaded in a 10X Genomics instrument and sequenced using the Illumina technology to an average of 65,000 reads/cells. **Results:** A total of ~28,000 single-cell transcriptomes were generated, of which ~78%, 3% and 18% corresponds to hepatocytes, cholangiocytes and non-parenchymal cells, respectively. Taking specific gene expression patterns related to zonated liver functions (i.e. glutamine metabolism, urea cycle, bile acid synthesis, xenobiotic metabolism and albumine production) as a reference, the single-cell transcriptomes obtained for hepatocytes have efficiently been organized along the porto-central axis, revealing the pericentral-, periportal- and midzonal-specific hepatocyte transcriptomes. Within the clusters of cholangiocytes and non-parenchymal cells, our results identify multiple subpopulations, including two distinct populations of hepatic stellate cells, of which the transcriptomic disparities hint to intriguing functional subspecializations, and in situ hybridization suggests their spatial zonation in the liver lobule. **Conclusion:** Our study provides a transcriptomic atlas of the human native liver at an unparalleled resolution and contributes to a better understanding of the heterogeneity of the cellular compartments that underlies the physiology of the human liver.