Multispectral Imaging Reveals Unique Macrophage Profiles Associated with Type of Liver Disease and Fibrosis Stage

Omar A. Saldarriaga1, Adam L. Booth1, Jared K. Burks, Netanya S. Utay2, MinKyung Yi3, Monique Ferguson4, Laura Beretta5 and Heather L. Stevenson1.

1Department of Pathology, University of Texas Medical Branch, Galveston, TX; 2University of Texas Health Science Center at Houston, Houston, TX; 3University of Texas Medical Branch, Galveston, TX; 4University of Texas MD Anderson Cancer Center, Houston, TX

Introduction

- Intrahepatic macrophages greatly impact the composition of the hepatic microenvironment, host immune response and development of fibrosis.
- Studies of human intrahepatic macrophages can be challenging for several reasons:
  - (i) they are difficult to isolate from human liver tissue
  - (ii) they become activated and change their phenotype when isolated or manipulated
  - (iii) in vitro and mouse models of HCV infection or fatty liver disease do not closely mimic the long-term, chronic infections that are observed in humans
- We are using spectral imaging microscopy with advanced imaging analysis software programs to analyze intrahepatic macrophages in situ in human liver biopsies.
- This platform is optimized for multiplex immunofluorescence staining of formalin-fixed paraffin-embedded tissues and does not compromise the hepatic architecture.
- This approach will allow us to gain an in-depth understanding of how variations in human hepatic macrophage profiles affect the host immune response and development of hepatic fibrosis.

Methods

1. Antibodies used to identify intrahepatic macrophages using this platform

<table>
<thead>
<tr>
<th>Macrophage: Parent cell markers</th>
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<tr>
<td>Tissue resident Kupffer cells</td>
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<tr>
<td>Systemic monocytes</td>
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2. Markers analyzed by level of expression on parent cells

<table>
<thead>
<tr>
<th>Classical (pro-inflammatory) macrophages</th>
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<tr>
<td>CD14+ / CD16-</td>
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<th>Intermediate macrophages</th>
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<td>CD14+ / CD16+</td>
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<th>Non-classical (anti-inflammatory) macrophages</th>
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<td>CD14+ / CD16+</td>
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3. Figure 1. Multiplex images obtained from staining FFPE liver biopsies from control patients compared to patients with three common chronic liver diseases (NASH, AIH and HCV). Biopsies were stained with the multiplex panel (CD68, CD163, Mac387, CD14, CD68, and DAPI) and representative images (20X) were acquired. Panel A: Fluorescent multiplex image after staining with the multiplex panel. Panel B: The multiplex images were then analyzed with Visiopharm phenotyping applications and each color represents a unique cellular phenotype. Panel C: The t-SNE plots highlight the unique profiles of macrophages that were identified in the livers of patients with chronic liver diseases versus controls. Panel D: Shows differences in the numbers of Mac387+ macrophages in the portal tracts and lobules in patients with either NASH, AIH or HCV, compared to control patients. Results shown are representative of three different patients with similar hepatitis activity scores (i.e., MNAI) and fibrosis stages (using the Ishak criteria). NASH: Nonalcoholic steatohepatitis; AIH: Autoimmune hepatitis; HCV: Hepatitis C virus. P tract: Portal tract; tSNE: t-distributed Stochastic Neighbor Embedding (tSNE) algorithm.

Fig. 1. Control

Fig. 2. CD68 - Opal 520, CD163 - Opal 650, Mac387 - Opal 690, CD14 - Opal 590, DAPI - Blue

Fig. 3. Unsupervised analysis shows multiple macrophage phenotypes in FFPE liver biopsies obtained from patients with NASH after staining with the macrophyte panel. (A) t-SNE plots use dimension reduction to facilitate visualization of macrophage marker expression. Cells with similar properties appear close together in a two-dimensional map and red (or “hot”) markers show cells with relatively more expression of that specific marker when compared to blue (or “cold”) markers, which indicate absent or minimal expression. (B) The phenotypic matrix algorithm identified 16 distinct macrophage phenotypes (k: 1-16) in one multiplex image obtained from a patient with NASH. Other cell populations, which likely include hepatocytes, lymphocytes and epithelial cells, appear blue in the phenotypic matrix map (k: 17-25).

Conclusions

- Spectral imaging microscopy is a powerful technique that enables quantification and identification of distinct intrahepatic macrophage phenotypes in situ in human FFPE liver tissue.
- The multiplex staining panel and imaging analysis software algorithms that we have developed allows these elusive cells to be studied in the context of intact hepatic architecture.
- The unique macrophage profiles that we have identified will be correlated with patient outcomes.

Acknowledgments

- Moody Endowment Research Award
- UT Systems Rising STARs Award
- Perkin Elmer: Kevin Mottenhead
- Visiopharm: Dr. Ben Freiberg and Dr. Alex Villa
- University of Michigan: Dr. Anindya Pao
- Rice University: Santhosh Krishnan

Contact Information

Heather Stevenson-Lerner, M.D., PhD
Assistant Professor, UTMB, Dept of Pathology
hlsteven@utmb.edu
Office: 409-772-8554

Omar A. Saldarriaga, DVM, PhD
Research Scientist, UTMB, Dept of Pathology
salda@utmb.edu
Office: 409-772-8375

Adam L. Booth, MD
Chief Resident, PDYO, UTMB, Dept of Pathology
aebuch@utmb.edu
Office: 936-318-1790