2415

An Integrated Approach on Immune-Cell Subtype Characterization Reveals Common Inflammatory Pathways in Nonalcoholic Steatohepatitis and Primary Sclerosing Cholangitis

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Introduction

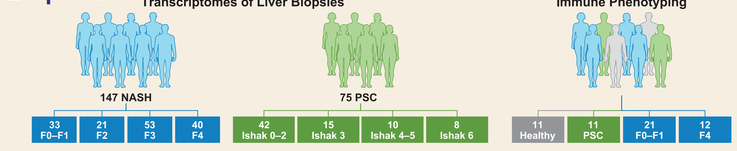
- Inflammation is a key driver for the progression of chronic liver diseases, which are characterized by parenchymal cell injury, immune-cell infiltration, and fibrogenesis[1]
- No pharmacologic treatment options exist for nonalcoholic steatohepatitis (NASH) and primary sclerosing cholangitis (PSC) at present
- Understanding the contributors and overlap in the immune landscape in NASH and PSC can provide a basis to develop new therapies

Objective

- To use novel technologies to dissect the immune response complexity in NASH and PSC to provide the basis for therapeutically modulating immune responses

Methods

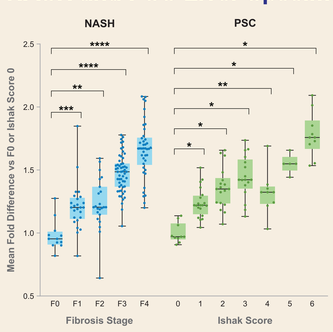
Biopsies



- Transcriptomes of liver biopsies from 147 NASH and 75 PSC patients were analyzed using 200 inflammation signature genes established by the Broad Institute (Cambridge, Massachusetts, USA)[2]
- To understand the alteration in immune profiling in nonlobular vs lobular areas in liver biopsies from healthy control subjects (n=11), and patients with PSC (n=11), and F0–F1 (n=21) and F4 NASH (n=12), a 12-plex UltiMapper® immunofluorescence assay (Ultivue, Inc., Cambridge, Massachusetts, USA)[3] was performed
- The spatial distribution of immune-cell subsets was characterized by 2 novel technologies: 12-plex UltiMapper technology and sequential multiplex immunostaining[4]
- For both technologies, unbiased analysis of whole-slide imaging from liver sections was performed

Results

Transcriptomic Analysis Revealed Similar Inflammation Signatures in NASH and PSC Liver Specimens as Disease Progressed

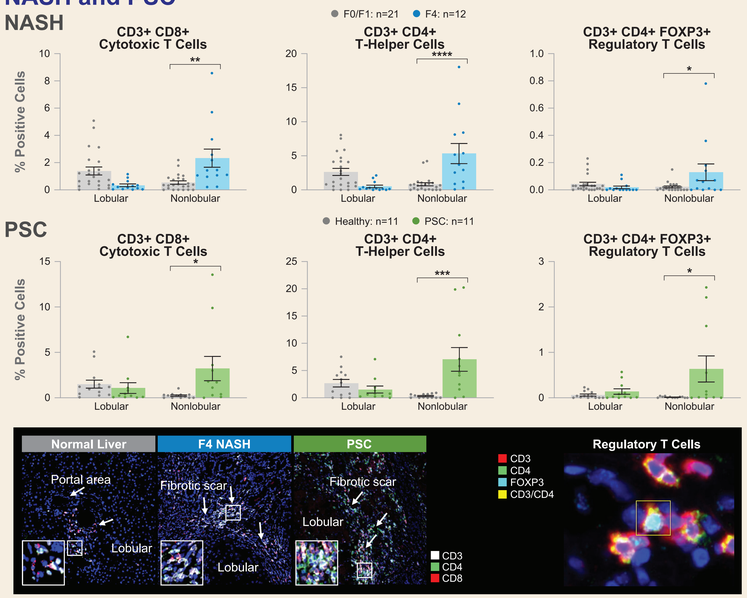


NASH (vs F0)				PSC (vs Ishak 0)						
F1	F2	F3	F4	1	2	3	4	5	6	
		2.19x	3.56x			2.96x	2.84x	5.25x	8.37x	*TIMP1*
		2.38x	4.76x			2.87x		4.02x	8.68x	*IL-7R*
		2.29x	3.88x			3.20x		3.85x	7.18x	*GPR132*
		2.20x	3.64x						9.01x	*KCNA3*
			2.79x					4.26x	7.71x	*BTG2*
			3.25x			2.94x		4.03x	4.59x	*HAS2*
		1.85x	2.99x			2.20x	1.96x	3.14x	5.00x	*NLRP3*
		2.03x	2.95x			2.00x	1.79x	2.49x	4.83x	*SEMA4D*
		1.73x	2.62x			2.22x	2.51x		5.36x	*PTGIR*
		2.34x	3.13x			2.32x	2.30x		4.88x	*CD48*
		1.83x	2.31x			2.93x			5.99x	*NOD2*
		3.01x	3.86x						5.00x	*HBEGF*
			2.56x						5.07x	*CD69*
		2.20x	2.11x						3.89x	*CXCL10*
			1.60x			2.51x		3.15x	3.73x	*MEFV*
			1.65x			2.56x	2.27x		3.26x	*RTP4*
										SELE
									6.68x	*IL-10*
		2.21x	3.35x			3.48x	3.57x	3.63x	8.94x	*LCK*
		2.19x	2.85x			3.82x		4.08x	7.29x	*PLAUR*
		1.95x	2.89x			3.18x	4.42x	3.77x	7.60x	*MYC*
		2.46x	3.46x			4.45x	4.80x	6.05x	9.04x	*LPAR1*
		2.79x	3.75x						8.55x	*LAMP3*
		2.46x	2.63x						4.80x	*CXCL9*
		2.68x	3.87x			4.68x	6.90x	8.89x	15.07x	*SLC7A1*
		4.64x	4.95x			5.48x	5.06x		7.20x	*CCRL2*
		3.68x	9.08x			3.02x	3.32x	5.15x	15.28x	*F3*
		3.09x	6.46x					5.49x	10.47x	*ITGB8*
		3.50x	4.95x			2.53x		6.45x	7.21x	*CCL2*
		6.33x	8.92x					13.94x	19.46x	*OLR1*
		19.64x	32.04x					42.11x	49.41x	*CCL20*
		9.60x	25.21x			14.10x		29.71x	55.09x	*CXCL6*
		8.89x	18.31x				12.37x		41.20x	*LIF*
		3.84x	7.56x			11.13x	11.37x	18.22x	43.70x	*SLAMF1*
		9.80x	28.36x			19.66x	24.95x	57.17x	102.31x	*GPC3*
			10.70x					85.65x	178.20x	*CXCL8*

*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001.

- RNA sequencing revealed that 64% and 47% of inflammation signature genes were upregulated in livers from patients with NASH cirrhosis and PSC Ishak 6, respectively, compared with healthy livers
- The highest upregulated genes in both diseases were C-C motif chemokine ligand-20 (*CCL20*), C-X-C motif chemokine ligand-6/8 (*CXCL6/8*), LIF interleukin-6 family cytokine (*LIF*), and signaling lymphocytic activation molecule family member-1 (*SLAMF1*)
- Upregulation in inflammation signature genes was observed as disease progressed and it correlated with fibrosis markers (α-smooth muscle actin: r=0.64, Enhanced Liver Fibrosis test [Siemens Healthcare GmbH, Erlangen, Germany]: r=0.60)

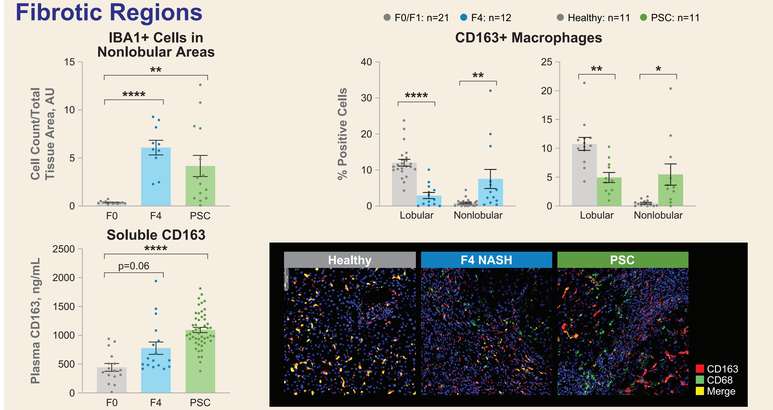
Infiltration of CD8 and CD4 T Cells Increased in Fibrotic Scars of NASH and PSC



*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001. CD, cluster of differentiation; FOXP3, forkhead box P3.

- In nonlobular areas of NASH livers, a 4.5-fold (p=0.001) increase in CD8+ T cells and 6.2-fold (p <0.001) increase in CD4+ T cells were observed compared with healthy livers; PSC liver samples also showed an 11.9-fold (p=0.03) elevation in CD8+ T-cell infiltration and 18.5-fold (p <0.001) increase in CD4+ T cells compared with healthy livers
- Regarding CD4 T-cell subsets, increased numbers of FOXP3+ regulatory T cells were found in F4 NASH (mean 0.13% [95% confidence interval -0.004, 0.26] vs F0/F1 (0.02% [0.008, 0.04]) and in PSC (0.64% [-0.007, 1.3] vs normal (0.01% [0.003, 0.02]) liver samples

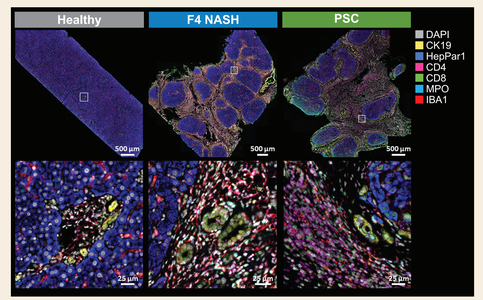
CD163+ Cells Decreased in Lobular Areas, But Increased in Fibrotic Regions



*p <0.05; **p <0.01; ***p <0.0001. IBA1, ionized calcium binding adaptor molecule-1.

- In the innate immune component, high infiltration of monocyte-derived macrophages (IBA1+ cells) was observed in nonlobular areas of F4 NASH and PSC patients compared with healthy livers
- CD163+ macrophages were 9-fold higher in livers with NASH fibrosis vs F0/F1
- PSC livers showed an 11-fold increase in CD163+ macrophages
- Decreases in CD163+ macrophages were observed in lobular areas of NASH (12% F4 NASH vs 3% healthy) and PSC (11% vs 5% healthy) livers
- Soluble CD163 was 1.4-fold (p <0.02) and 2.5-fold (p <0.001) higher in NASH and PSC, respectively, vs healthy livers

Immune-Cell Infiltration in NASH and PSC



CK19, cytokeratin-19; DAPI, 4',6-diamidino-2-phenylindole; HepPar1, hepatocyte paraffin 1; MPO, myeloperoxidase.

Conclusions

- NASH and PSC share common inflammation signature genes that correlate with stage of the disease and fibrosis markers
- The novel multiplex technologies allowed a broader understanding of the liver immune microenvironment, showing similar trends toward increases in CD4, CD8, and regulatory T cells, and monocyte-derived macrophages
- Increases in the infiltration of CD163-positive monocytes and plasma levels of soluble CD163 were also observed during progression of NASH and PSC
- Together, these approaches provide further disease understanding and enable therapeutic discoveries to treat liver diseases

References: **1.** Schuster S, et al. Nat Rev Gastroenterol Hepatol 2018;15:349-64; **2.** Liberzon A, et al. Cell Syst 2015;1:417-25; **3.** Manesse M, et al. Methods Mol Biol 2020;2055:585-92; **4.** Guillot A, et al. Cancers (Basel) 2020;12:2449.

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